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THE CYTOLOGICAL BEHAVIOUR AND MODE OF TRANSMISSION OF ACCESSORY CHROMOSOMES IN PLANTAGO SERRARIA

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I. INTRODUCTION

PLANTAGO SERRARIA is a herb which has its main distribution around the Mediterrianean Sea. BÖCHER, LARSEN and RAHN (1955) consider the species to be a pauciennial. However, from the observations made during this investigation [1] it seems probable that *Plantago serraria* is a perennial. The chromosome number of *Plantago serraria* is 2n=10 and it thus belongs to the group of species referred to the section *Coronopus*. According to BÖCHER, LARSEN and RAHN the section *Coronopus* consists of two natural groups of species namely the *coronopus* and the *serraria* groups. The latter group, including *P. serraria*, differs from the *coronopus* group in having larger chromosomes.

Beside plants with the normal 10 chromosomes BÖCHER, LARSEN and RAHN (1955) found some individuals of *P. serraria* with accessory chromosomes. The following study was made in order to obtain more information about these accessory chromosomes, especially their mode of transmission.

The material was obtained from Portugal and consisted of seeds which were collected from a natural population growing along roadsides at Sacavem near Bobadela in the province of Estremadura. From this seed sample 315 plants were raised and their chromosome number determined. From Table 1 is seen that 19 plants (approximately 6 per cent) had one accessory chromosome each. Since BÖCHER, LARSEN and RAHN found the same type of accessory chromosome from 5 out of 8

[1] Seeds were sown in the late winter or early spring of 1957, the plants flowering during the next summer. The following winter the plants were kept in the greenhouse and flowered again in the spring and summer of 1958. The plants were very vigorous after the second flowering period during the winter of 1958—1959.

TABLE 1. *Frequency of accessory chromosomes in a natural population of P. serraria from Portugal.*

No. of plants with respective no. of acc. chr.		% plants with acc. chr.
0	1	
295 [1]	19	6

[1] One plant was triploid.

strains studied by them, it seems as if plants with accessory chromosomes are relatively common in the species.

II. METHODS

For ordinary chromosome counts root-tips were fixed in chrome-acetic-formalin, sectioned at 12 μ and stained with crystal violet. For a more detailed study on the morphology of the chromosomes root-tips squashes were used. In this case the root-tips were pretreated with oxiquinolin, fixed in metanol and propionic acid (3:1) and stained with Feulgen and chrome-hematoxylin, according to MELANDER and WINGSTRAND (1953). (For valuable advice concerning the squashing technique I am indebted to Dr. G. ÖSTERGREN.)

For the study of meiosis, sections as well as squashes have been used. For sectioning the buds were pretreated in metanol-propionic acid (3:1) for 2 mins. and fixed in chrome-acetic-formalin. The squashes were made from fresh material as well as from buds fixed in metanol-propionic acid, and in both cases they were stained in aceto-orcein.

For the study of the transmission of the accessory chromosomes, crosses were made by isolating the parental plants in isolation cages in the greenhouse. *Plantago serraria* is normally wind pollinated and the seed-setting has generally been satisfactory. To obtain some information on the degree of self-fertilization one or two panicles of the parental plant were isolated in a pollen-proof bag. Generally these isolated panicles set only a few seeds. However, one plant set a large number of seeds on isolation and the cross in which this plant took part was therefore discarded.

III. CYTOLOGICAL STUDIES

1. Mitosis

The size and shape of the five ordinary chromosomes of *Plantago serraria* are illustrated in Fig. 1. Four of the chromosomes have a sub-

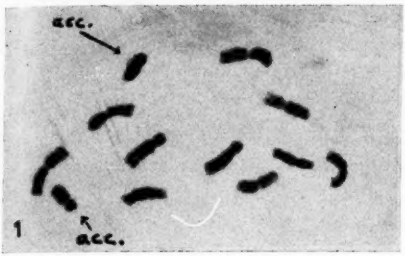

Fig. 1. Somatic metaphase from a plant with 2n=10+2 acc. chr. — Fig. 2. Somatic prophase in a plant with 2n=10+2 acc. chr. showing positive heteropycnosis of the accessories. Also the ordinary chromosomes show positive heteropycnosis on both sides of the centromeres. — Fig. 1, c:a 800×; Fig. 2, c:a 600×.

median and one a subterminal centromere. The two largest chromosomes are probably the SAT-chromosomes and have secondary constrictions which, however, are often difficult to detect, at least when the material is fixed in chrome-acetic-formalin.

The accessory chromosomes are relatively large and have a submedian centromere. Their length in somatic metaphase plates is about $^3/_4$ of the average of the ordinary chromosomes (Fig. 1). They are positively heteropycnotic in the somatic prophase. The ordinary chromosomes also have heterochromatic segments, especially near the centromeric region, whereas the accessory chromosomes appear to be heterochromatic along their full length (Fig. 2).

To study the constancy of the accessory chromosomes within the individual plants altogether 114 somatic metaphase plates from 5 plants were examined. Only exceptionally was any variation in number of accessories found between different cells from the same plant. Furthermore, the same number was always found in the pollen mother cells as in the root-tips. It can thus be concluded that the number of accessory chromosomes in *Plantago serraria* is constant within the plant.

2. Meiosis

The ordinary chromosomes often form four rod and one ring bivalent at metaphase I. However, univalent chromosomes are rather often seen and they probably consist of one of the shortest chromosome pairs which may have failed to form chiasmata.

The accessory chromosomes have not shown any tendency to pair with the ordinary chromosomes and, as will be described below, their

meiotic behaviour also deviates markedly. At pachytene, diplotene and diakinesis the accessory chromosomes are easily identified because of their strong positive heteropycnosis.

The pairing of the accessory chromosomes during early meiotic prophase has been studied in a few cells only. It has been found, however, that, although the accessories usually are paired at pachytene when two are present, unpaired accessories are also rather frequent. The accessories have a tendency to be placed peripherally in the nucleus at early prophase of meiosis, as was also observed by BÖCHER, LARSEN and RAHN (1955). At diakinesis a better estimate of the pairing ability of the accessories was obtained. In one plant with two accessory chromosomes they were associated in 32 cells out of 74, associations end to end and side by side being equally frequent. In this case, as in *Centaurea scabiosa* (FRÖST, 1956), it is unlikely that the accessory chromosomes are associated by chiasmata since no true bivalents have been observed with certainty at metaphase I.

At first metaphase the univalent accessory chromosomes are very often located close to or at the poles (Figs. 6—7). In but few cases, only, have the accessories been seen in the equatorial region. The accessory chromosomes have never been seen to form true bivalents at metaphase I. Sometimes in plants with two accessory chromosomes bivalent-like associations were observed but, as has been pointed out, it is not probable that these configurations represent bivalents associated by chiasmata. More probably they are held together by stickiness or they may simply be lying close to one another, without any real association. They may originate from the associations of accessory chromosomes seen during the meiotic prophase which have thus not yet separated. However, as is the case with the univalent accessories these "bivalents" are located close to one of the poles and not in the equatorial region (Fig. 7). With regard to their ability to move undivided to the poles before the start of anaphase I the accessory chromosomes in *Plantago*

Fig. 3. Pachytene from a plant with 1 acc. chr. showing positive heteropycnosis. — Fig. 4. Diplotene from a plant with $2n=10+2$ acc. chr. showing unpaired accessories. — Fig. 5. Diakinesis in a plant with $2n=10+2$ acc. chr. The accessories are associated with each other forming one bivalent. — Fig. 6. Metaphase I in a plant with $2n=10+1$ acc. chr. The accessories in all three cells are localized close to the poles. — Fig. 7. Metaphase I in a plant with $2n=10+2$ acc. chr. The accessories are connected with each other by a thin chromatin thread and have localized themselves close to the pole. — Fig. 8. Anaphase I in a plant with $2n=10+2$ acc. chr. The accessories are situated close to the poles and will undivided be included in the anaphase groups. — Fig. 3, c:a 1000×; Figs. 4—5, 7—8, c:a 600×; Fig. 6, c:a 400×.

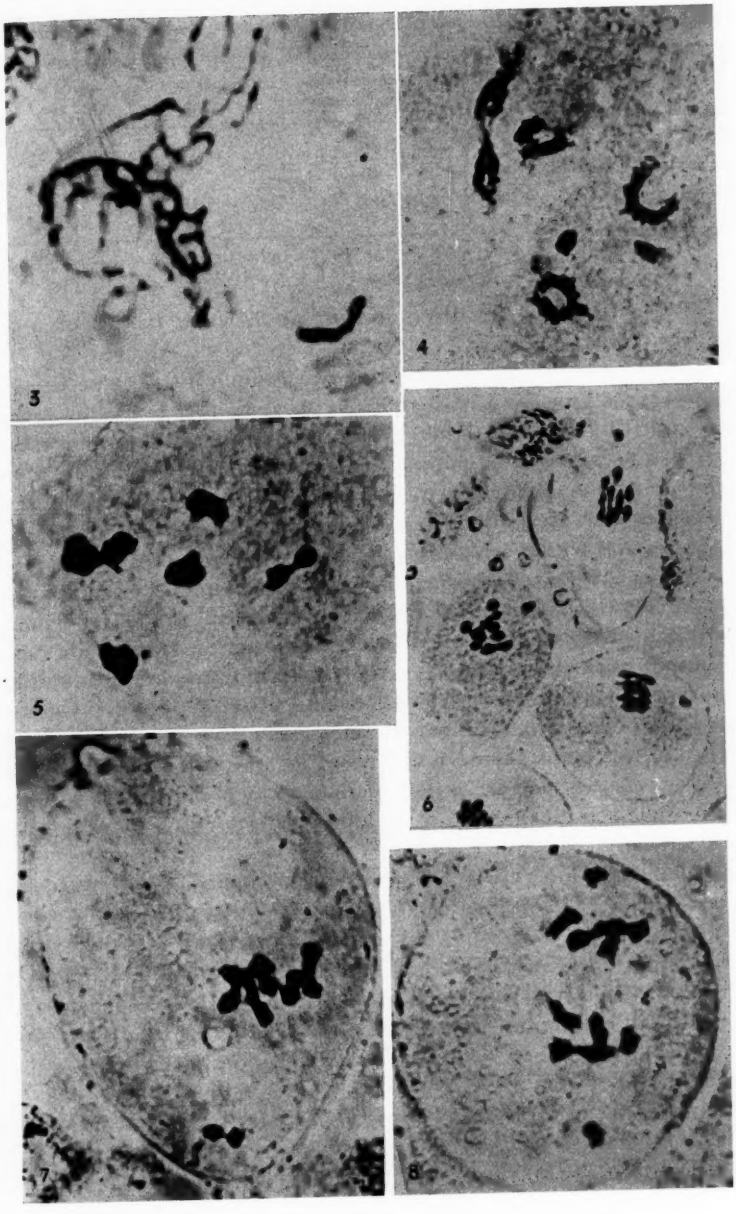
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TABLE 2. *Position of univalent accessory chromosomes at metaphase I.*

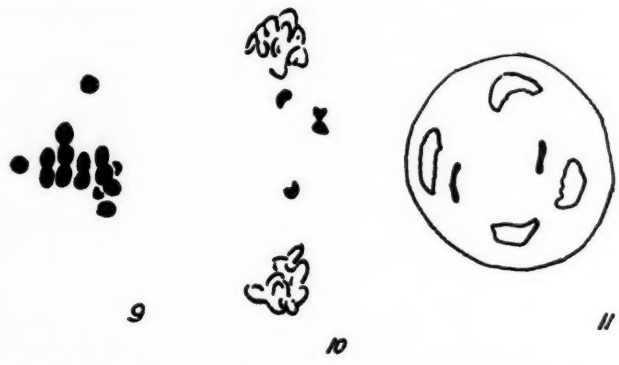
Plant No.	Number of acc. chr.	On the same side of the equat. region	On opposite sides of the equat. region	Total number of cells
10 Bp—2	2	16	19	35
10 Bp—4	2	50	52	102
10 Ap—5	2	41	18	59
10 Ap—6	2	51	46	97
Total		158	135	293

serraria behave like univalent accessories in some species of grasshoppers (WHITE, 1954).

In plants with two accessory chromosomes their distribution between the poles at M I has been studied (Table 2). From the observations made by BÖCHER, LARSEN and RAHN (1955) there appeared to be a preference for a 2:0 distribution as opposed to 1:1. BÖCHER, LARSEN and RAHN suggest that this might be a mechanism for numerical increase of the accessories. However, although there would be an increase in the number of gametes with 2 accessories the number of gametes with no accessories would increase correspondingly and unless other circumstances, too, influence their transmission, such a mechanism would not result in an average numerical increase of the accessory chromosomes. However, the author has not been able to verify BÖCHER, LARSEN and RAHN's observations in the present material. In one plant, a tendency to a 2:0 distribution was obtained, but as only early metaphase stages were studied in this plant, the deviating result may be due to the orientation of the accessories not yet being completed. Such a tendency to a 2:0 distribution at pro-metaphase I might be due to sticky associations during the meiotic prophase.

Since the accessory chromosomes are located at the poles already before anaphase I sets in they will be included without division in one of the polar groups (Fig. 8). In no case have divided univalents of the accessory chromosome type been observed, and lagging undivided univalents are but rarely seen at anaphase I. In the few cases where an accessory chromosome is left between the poles at A I there is a tendency to splitting, but the daughter chromatids appear never to separate completely.

The later stages of meiosis are more difficult to study. At metaphase II all the chromosomes are clumped together on the equatorial plate and they are difficult to identify. However, chromosomes outside the



Figs. 9—11. Meiosis in a triploid plant without accessory chromosomes. — Fig. 9. Metaphase I, $2_{III}+3_{II}+3_{I}$. — Fig. 10. Late anaphase I showing one univalent which has divided and one univalent which prepares for division. — Fig. 11. Late anaphase II showing lagging daughter univalents. — Figs. 9, 11, c:a 600×; Fig. 10, c:a 750×.

plate are found but rarely, and the accessories are thus obviously generally localized in the metaphase plates.

At anaphase II the accessories seem to divide in the normal manner. At late anaphase II and telophase II a low frequency of lagging chromosomes has been observed, but it is difficult to decide whether they are accessories or ordinary chromosomes. At the tetrad stage there is a very low frequency of micronuclei and even if all of these were caused by accessory chromosomes they indicate that the meiotic elimination is very slight.

3. Univalents of ordinary chromosomes during meiosis

In order to compare the meiotic behaviour of the univalent accessory chromosomes with that of univalents of the ordinary chromosome complement meiosis of a triploid plant has been studied. This plant had no accessory chromosomes. The observations gave clear evidence of a marked difference in the meiotic behaviour between ordinary univalents and univalent accessories. At metaphase I univalents from the normal complement are distributed over the spindle more or less at random. They are found close to or in the equatorial region as often as at the poles. At anaphase I they may go undivided to the poles but they frequently divide (Fig. 10). As a result of this, there are found daughter chromosomes lying outside the plate at M II and laggards at A II

TABLE 3. *Number of accessory chromosomes in progeny plants from crosses of the type 10×(10+1 acc. chr.).*

Parents		F_1-plants			Number of plants	$\bar{x}$ acc. chr. per plant
Plant numbers	Number of acc. chr.	Number of acc. chr. 0	1	2		
P—44×P— 19	0×1	25	19		44	0.43
P—22×P— 76	0×1	14	18	1	33	0.61
P—15×P— 76	0×1	28	18	1	47	0.43
P—33×P—131	0×1	19	16		35	0.46
P— 1×P—131	0×1	25	21		46	0.46
Total	0×1	111	92	2	205	0.47

(Fig. 11). At the tetrad stage micronuclei are common, indicating a high degree of elimination.

IV. THE INHERITANCE OF THE ACCESSORY CHROMOSOMES

The inheritance of the accessory chromosomes was studied in altogether 17 different cross combinations. Seed setting has generally been satisfactory but as seen from Tables 3 and 4 it has not been possible always to analyse reciprocal crosses. This was due to the poor germination rather than to a shortage of seed.

1. Crosses of the type 10×(10+1 acc. chr.)

The result of these crosses are given in Table 3. The following numbers and categories of plants were obtained: 111 without, 92 with one and 2 with two accessory chromosomes. There is thus no tendency to a numerical increase of the accessories. Instead there is a certain loss which is very probably the result of some meiotic elimination. The meiotic loss, however, is very low compared with corresponding values for univalent accessory chromosomes in several other species e.g. *Secale cereale* (MÜNTZING, 1945). This result is in agreement with the observations made at meiosis where indications were obtained that the meiotic elimination was very low. The low meiotic elimination was assumed to depend on the peculiar ability of the accessories to become localized close to one of the poles at metaphase I and to pass undivided through anaphase I.

The two exceptional plants with two accessory chromosomes might be the result of non-disjunction at some stage of meiosis or mitosis, but

TABLE 4. *Number of accessory chromosomes in progeny plants from crosses of the type (10+1 acc. chr.)×10.*

Parents		F_1-plants			
Plant numbers	Number of acc. chr.	Number of acc. chr. 0	1	Number of plants	$\bar{x}$ acc. chr. per plant
P— 19×P—44	1×0	16	33	49	0.67
P—131×P—33	1×0	3	10	13	0.77
P—131×P— 1	1×0	2	13	15	0.87
P— 19×(P—28, P—44)	1×0	24	57	81	0.70
P— 76×(P—22, P—15)	1×0	8	15	23	0.65
P—118×(P—40, P—32)	1×0	23	55	78	0.71
Total	1×0	76	183	259	0.71

their low frequency makes it very unlikely that they are due to a real mechanism for numerical increase of the accessory chromosomes.

The data in Table 3 indicate differences between crosses with respect to the degree of elimination of the accessories. A test of heterogeneity, however, showed these differences to be non-significant ($\chi^2=8.3230$) but because of the small number of crosses analysed this result should not be considered conclusive.

2. Crosses of the type (10+1 acc. chr.)×10

As seen from Table 4 six crosses of the type (10+1 acc. chr.)×10 were analysed. If it is assumed that the accessory chromosomes on the female side are distributed in the same manner as on the male side i.e. at random and with about ten per cent average meiotic elimination, one should expect in F_1 54 per cent of plants without and 46 per cent of plants with one accessory chromosome. However, calculated on the sum of daughter plants (cf. Table 4) there are 29 per cent plants without and 71 per cent plants with one accessory chromosome. The ratio 29:71 differs significantly from the 54:46 ratio obtained when the accessory chromosome was transmitted through the pollen ($\chi^2=29.26$, $P<0.001$).

The surplus of plants with one accessory chromosome cannot depend on any post-meiotic process of non-disjunction of the same kind as in *Secale cereale* (MÜNTZING, 1945; HÅKANSSON, 1948), since such a process should result in a high frequency of plants with 2 accessory chromosomes. Nor it is likely that the excess of plants with one accessory chro-

mosome is due to self-fertilization of the mother plants. All the plants were tested with respect to self-fertility and only one or two seeds were obtained after isolation. Furthermore, if self-pollination were responsible for the deviation obtained, one would expect a considerable number of plants with two accessory chromosomes.

Instead it is very likely, that the accessories are preferentially included in the egg cell as the result of a directed distribution at meiosis in a way similar to that found in *Lilium callosum* (KAYANO, 1956, 1957). In this species, at the first meiotic division of the E.M.C. the accessory chromosomes are preferentially included in that dyad cell which gives rise to the embryo-sac and thus the egg cell. The same has been found for the accessory chromosomes in *Trillium grandiflorum* (RUTISHAUSER, 1956).

(The data in Table 4 indicate a variation between different crosses in the mean values of the accessories. However, the χ^2-value obtained ($\chi^2=2.6677$), with 5 degrees of freedom, gave a P-value between 0.80 and 0.70.)

3. Crosses of the type 10×(10+2 acc. chr.), (10+2 acc. chr.)×10 and (10+1 acc. chr.)×(10+1 acc. chr.)

One cross of the type *10×(10+2 acc. chr.)* has been studied. The data from this cross are given in Table 5. The meiotic studies in plants with two accessories showed that nearly always the accessories are univalent at metaphase I. This is also reflected by the irregular distribution of chromosome numbers among the F_1-plants.

It was considered above (p. 196) whether the accessories segregate independently of each other or whether there is a tendency to a 2:0 distribution at anaphase I. Assuming independent segregation the following types and frequencies of gametes should be produced in a plant with two accessory chromosomes: without, with one, and with two accessories in the ratio 1:2:1. Thus, in the present cross, where no meiotic elimination seems to occur, the following frequency of the daughter plants would be expected: 11.75 with no accessory chromosome, 23.50 with one and 11.75 with two. When these expected values are compared with those actually obtained there is, as seen from Table 5, a good agreement ($\chi^2=1.5531$ and $0.50 > P > 0.30$). The number of progeny plants in this cross is rather low, however, and the result in itself may not be conclusive. At any rate the result is in agreement with the cytological observation of independent segregation of the accessories during microsporogenesis and supports this interpretation.

TABLE 5. *Number of accessory chromosomes in progeny plants from crosses of the types 10×(10+2 acc. chr.) and (10+2 acc. chr.)×10.*

Parents		F_1-plants				Number of plants	$\bar{x}$ acc. chr. per plant
Plant number	Number of acc. chr.	Number of acc. chr. 0	1	2	3		
1 Bp—19×(10 Bp—10, 10 Ap—10, 10 Bp—4)	0×2	9	23	15		47	1.13
Expected values (X^2=1,5531, 0,50>P>0,30)		*11.75*	*23.50*	*11.75*		*47*	
10 Bp—13 (1 Ap—24, 2 Ap—5, 1 Ap—22)	2×0	4	22	24	2 [1]	52	
10 Ap—9 (1 Ap—13, 1 Ap—3, 2 Ap—6)	2×0	2	9	5		16	
10 Ap—5 (1 Ap—49, 1 Ap—32, 2 Ap—13)	2×0	2	3	13		18	
Total	2×0	8	34	42	2	86	1.44
Expected values (X^2=0,1476, 0,95>P>0,90)		*7.2326*	*35.4148*	*43.3526*		*86*	

[1] May depend on self-fertilization.

Three crosses *(10+2 acc. chr.)×10* have been analysed (Table 5). Unfortunately, the number of daughter plants is low because of poor germination. However, the material is sufficient to demonstrate the importance of the univalent character of the accessories in *Plantago serraria* for the effectiveness of the mechanism of preferential segregation. Assuming that this preferential segregation occurs to the same extent in plants with two accessory chromosomes as was observed in plants with one, and that the accessories are distributed independently of each other, the following types and frequencies of zygotes should be expected; 0.08 with no accessory chromosome, 0.41 with one and 0.50 with two. As may be seen from Table 5 there is no significant difference between the expected and obtained values for the various classes.

If, on the other hand, the pairing behaviour of the accessories had been quite regular, all the daughter plants should have had a single accessory chromosome, only. Thus, the mechanism of preferential segregation in *Plantago serraria* appears to be as effective in plants with two accessory chromosomes as in those with one. This is not the case in *Lilium callosum* (KAYANO, 1956), where pairs of accessories rather regularly form bivalents at metaphase I and where the preferential segregation occurs only when the accessories are univalent.

Two crosses of the type *(10+1 acc. chr.)×(10+1 acc. chr.)* have been analysed (Table 6). Assuming that gametes with none and with one accessory chromosome are produced in the ratio 0.54:0.46 on the male side (cf. Table 3) and in the ratio 0.29:0.71 on the female side (cf. Table 4), zygotes with none, with one and with two accessories should be produced in the ratio 0.1566:0.5168:0.3266. As is evident from Table 6 the expected frequencies agree fairly well with those actually obtained. In a cross of the type mentioned one can also get an idea of the increase in the average number of accessories owing to the preferential segregation on the female side. The average number of accessory chromosomes in the progeny is about 14 per cent higher than the mean of the parents. The mechanism is thus effective enough to compensate for the meiotic loss on the male as well as on the female side and must thus be considered an important factor for the maintenance of the accessories in natural populations.

V. POLLEN FERTILITY

It has been reported (PALIWALL and HYDE, 1958) that plants with a single accessory chromosome in *Plantago coronopus* always are com-

TABLE 6. *Number of accessory chromosomes in progeny plants from crosses of the type (10+1 acc. chr.)×(10+1 acc. chr.).*

Parents		F_1			Number of plants	$\bar{x}$ acc. chr. per plant
Plant numbers	Number of acc. chr.	Number of acc. chr. 0	1	2		
P—108×P—112	1×1	6	18	10	34	1.12
1 Ap—7×4 Bp—24×4 Bp—29×1 A—33	1×1	28	75	51	154	1.15
Total	1×1	34	93	61	188	1.14
Expected values (X^2=0,8866, 0,70>P>0,50)		*29.4408*	*97.1584*	*61.4008*	*188*	

TABLE 7. *Pollen fertility in progeny plants from a reciprocal cross.*

Number of acc. chr.	Percentage of good pollen 0	50	60	70	80	90	100	Number of plants	$\bar{x}$ %
0		1	1	4	5	22		33	88.9
1	1			3	13	32		49	89.2
Total								82	

pletely male sterile. To investigate the influence of the accessory chromosomes on male fertility in *Plantago serraria* pollen samples were taken from plants originating from a reciprocal cross of the type $10\times(10+1$ acc. chr.). The pollen was mounted in lacto-phenol-fuchsin.

As is seen from Table 7 plants with one accessory chromosome have, on an average, about the same percentage of good pollen as plants without accessories. One male sterile plant was obtained among the plants with one accessory chromosome, but since the plant is a single one out of 49 studied, it is very unlikely that the sterility has anything to do with the presence of the accessory chromosome. On the contrary, it must be supposed that the influence of one accessory chromosome on pollen fertility in *Plantago serraria* is very small.

VI. DISCUSSION

Concerning the inheritance of the accessories in *Plantago serraria* it was shown that there is a difference between the male and the female side. On the male side no mechanism of numerical increase was found. Instead, there was observed a definite but very low degree of meiotic elimination. The distribution of the accessories to the male gametes seems to be quite at random. On the female side, on the other hand, a significant deviation from a random distribution was obtained, i.e. in the F_1-generation from a cross of the type $(10+1$ acc. chr.$)\times 10$ there was a marked surplus of plants with one accessory in comparison to plants with no accessory chromosomes. Thus, a directed distribution of the accessories appears to occur at some stage of embryo sac development which causes the accessories to segregate preferentially to a cell producing the egg cell.

The same type of transmission of the accessories on the female side has earlier been demonstrated in *Trillium grandiflorum* (RUTISHAUSER, 1956) and *Lilium callosum* (KAYANO, 1956, 1957). In *Lilium callosum* (KAYANO, 1957) it was possible to study the behaviour of the accessories

during the course of the embryo sac development. It was demonstrated that a directed distribution of the accessories occurs during the first meiotic division of the E.M.C. The accessory chromosomes in most cases localized themselves on the micropylar side of the plate at metaphase I and moved in about 80 per cent of the divisions to the micropylar pole at anaphase I. As the development of the embryo-sac in *Lilium callosum* is of the *Fritillaria*-type, it is the micropylar dyad cell that gives rise to the embryo-sac. It is probable that a similar mechanism is at work also in *Plantago serraria*. However, as the development of the embryo-sac in *Plantago serraria* is of the normal type, the localization and the distribution of the accessories during the first meiotic division ought to be directed towards the chalazal side of the E.M.C., as it is the chalazal dyad cell that develops into an embryo-sac and produces the egg cell. In *Trillium grandiflorum* the meiotic mechanism of directed distribution ought to work in the same direction as in *Plantago serraria*, as the embryo-sac development in *Trillium* is of the *Allium*-type.

The factors responsible for such a meiotic mechanism for numerical increase of the accessory chromosomes on the female side probably result from an interaction of special conditions in the E.M.C. with special properties of the accessory chromosomes themselves. In *Lilium callosum* the assymetry of the spindle during the first division of the E.M.C. has been considered to be an important factor (KAYANO, 1957). The direction of the non-disjunction of the accessories towards the generative nucleus during the first pollen-mitosis in the grasses has also been supposed to be due in part to an assymetry of the spindle (ÖSTERGREN, 1947).

Further comparison with the process of non-disjunction in the grasses directs the attention to the centromere or the centromeric region of the accessories. The fact that univalent accessory chromosomes in *Plantago serraria* as well as in *Lilium callosum* and *Trillium grandiflorum* move undivided to the poles during anaphase I might depend on special properties of the centromere. The ability of the accessories to remain undivided during the first meiotic division must be considered to be absolutely necessary if the directed distribution of the accessories on the female side occurs during this division.

The remarkable ability of the accessories in *Plantago serraria* to localize themselves at, or rather close to the poles at metaphase I may also be mentioned in discussing possible special functions of their centromere. One may suggest that such a localization of the accessories together with their ability to remain undivided during anaphase I would

reduce the chance of their meiotic elimination. It is also possible that the presumed directed distribution at anaphase I during female meiosis may in some way be influenced by this capacity of polar localization.

The accessory chromosomes in *Plantago serraria* were found to be clearly heterochromatic, and in this respect they are similar to the accessories of several other species but differ from the accessories of *Trillium grandiflorum*, which according to RUTISHAUSER (RUTISHAUSER, 1956) are clearly euchromatic. This difference should be emphasized, because one of the factors of importance to the process of non-disjunction in the grasses seems to be special properties of heterochromatic segments in the accessory chromosomes (MÜNTZING, 1954; LONGLEY, 1956; BOSEMARK, 1956). The meiotic mechanism of directed distribution, on the other hand, functions also with euchromatic accessory chromosomes.

No case of bivalents formed by the accessories have been seen in *Plantago serraria* during metaphase I. In *Lilium callosum*, on the other hand, the accessory chromosomes very frequently form bivalents. The causes of such differences in pairing ability between accessory chromosomes in different species have been discussed in an earlier paper (FRÖST, 1956). The reason for the weak pairing in the case of *Plantago serraria* may be connected with the heterochromatic nature of the accessories. As has been suggested by WHITE (1954) heterochromatic segments prevent the formation of chiasmata. However, the lack of bivalent formation in *Plantago serraria* is of importance for the effectiveness of the preferential segregation. This fact has been discussed in connection with the presentation of the results from the crosses of the type (10+2 acc. chr.)×10 (p. 202).

The preferential segregation in *Plantago serraria* as well as in *Lilium callosum* and *Trillium grandiflorum* results in an increase in the number of accessory chromosomes from one generation to the next in spite of a certain degree of meiotic elimination. *Plantago serraria* is the first dicotyledonous species where a special mechanism for numerical increase of the accessory chromosomes has been found, but it should be remembered that, so far, the inheritance of accessory chromosomes have been studied only in a few dicotyledonous species. In *Centaurea scabiosa* (FRÖST, 1957) no evidence of any mechanism of numerical increase of the accessories was obtained. The very small accessory chromosomes of this species are always found as univalents as is the case of *Plantago serraria*, but unlike the accessories of *P. serraria* they often divide at anaphase I. Also they are distributed more or less at random to the gametes on the male as well as on the female side. *Caltha*

palustris is another case in which no evidence is found of a mechanism of numerical increase of the accessories (REESE, 1954).

In other dicotyledonous species investigated e.g. *Crepis syriaca* (CAMERON, 1934), *Oenothera* (CLELAND, 1951), *Godetia viminea* (HÅKANSSON, 1949), the data on transmission of the accessories are very scarce and insufficient for safe conclusions. However, in *Xanthisma texanum* (BERGER and WITKUS, 1954; WITKUS, LOWERY and BERGER, 1955; BERGER, MCMAHON and WITKUS, 1955), where the accessory chromosomes occur in the shoots but are eliminated from the primary roots, careful cytological studies did not indicate any processes neither on the male nor on the female side which could be interpreted as a mechanism of numerical increase of the accessory chromosomes.

On the other hand, such a mechanism seems to be the general rule for the accessory chromosomes in the grasses (BOSEMARK, 1957; MÜNTZING, 1958). The process which causes this numerical increase is the well-known mechanism of directed non-disjunction during the first or second pollen mitosis. In *Secale cereale* there is also a directed non-disjunction of the accessories at the first mitosis in the embryo sac (HÅKANSSON, 1948). The accessory chromosomes in species belonging to the family *Graminae* according to BOSEMARK (BOSEMARK, 1957) seem to form a fairly homogenous group. However, a certain variation between different species occurs, and the mechanism of directed non-disjunction of the accessories does not occur in all the grass species, exceptions being represented by *Poa alpina* (MÜNTZING, 1954; HÅKANSSON, 1954), *Poa trivialis* and *Phleum nodosum* (BOSEMARK, 1957). In these species no other mechanism of numerical increase of the accessories has been found.

It is evident that different ways of transmitting the accessories from one generation to the next have evolved in different plant species. Which way is the most common one is impossible to decide because of the relatively few species investigated in this respect. For the same reason all types of transmission of accessory chromosomes have certainly not yet been detected. It is, however, likely that the process of directed non-disjunction of the accessories during some of the postmeiotic divisions is the most frequent type in the grasses (as it has been found in 10 out of 13 investigated species). It also seems probable that the process of directed distribution of the accessories during meiosis, now known to occur on the female side in *Lilium callosum*, *Trillium grandiflorum* and *Plantago serraria* may be widespread, since it has been found in species belonging to two different families representing monocotyledons as well

as dicotyledons. As it is probable that about the same mechanism is responsible for this process in each case, it is interesting to note that evolution has taken the same way in so widely different species.

SUMMARY

(1) The cytological behaviour and mode of transmission of the accessory chromosomes in *Plantago serraria* were studied.

(2) The accessory chromosomes are rather big with a sub-terminal centromere and are clearly heterochromatic. The number of accessory chromosomes is constant within the plant.

(3) The meiotic behaviour of the accessory chromosomes deviates markedly from that of the ordinary chromosomes. They always seem to be univalent at metaphase I and localize themselves close to or at the poles. The polar localization causes the accessories to be included without division in one of the polar groups at anaphase I.

(4) In the mode of inheritance of the accessory chromosomes there was a difference between the male and the female side.

(5) On the male side there was not found any special mechanism of numerical increase. Instead there was a small loss of accessories from one generation to the next.

(6) On the female side on the other hand there was a mechanism of numerical increase of the accessory chromosomes. A directed distribution of the accessories appears to occur at some stage of the embryo-sac development which causes them to segregate preferentially to a cell later on giving rise to the egg cell.

(7) The frequencies of gametes in plants with two accessories indicate that the accessories segregate independently of each other.

(8) The univalent character of the accessories was found to be of importance for the effectivenss of the meiotic mechanism of directed distribution.

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CREPIS PANNONICA AND CREPIS CONYZAEFOLIA — TWO MORE SPECIES HAVING ACCESSORY CHROMOSOMES

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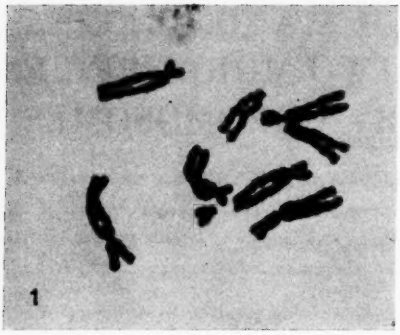
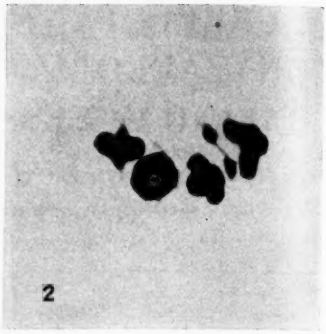
IN certain respects the genus *Crepis* has been subjected to a rather extensive cytological study. Thus, the chromosome number and the main features of the chromosome morphology have been investigated in about 115 species (BABCOCK, 1947 a and b; DARLINGTON and WYLIE, 1955). The interest of the investigators has usually been focused on problems of evolution and taxonomical relationship and in most cases presumable but few individuals of each species or variety have been investigated. In some materials, however, attention has been paid to the occurrence of a chromosomal variation between different individuals of the same species and a certain numerical variation has been reported. In most cases the variation appears to be caused by polyploidy and aneuploidy, as in *Crepis vesicaria*, where diploid and tetraploid races hybridize (BABCOCK, 1947 b, p. 480) and in *C. biennis* where the numerical variation may be connected with the meiotic irregularities that often occur in high polyploids. In *C. sibirica* the occurrence of supernumerary chromosomes was attributed to "fragmentation or translocation or a combination of both" (SWEZY, 1935). As far as we know, only one species has been clearly demonstrated to contain accessory chromosomes (B chromosomes), *viz. Crepis syriaca* (CAMERON, 1934). In order to detect the occurrence of accessory chromosomes it is often necessary to investigate many individuals of the same species, however. For this reason it appears probable that chromosomes of this kind may occur also in some other species of *Crepis* although they have not yet been reported.

During a study of the chromosome conditions of a number of *Crepis* species cultivated by us for another investigation we found two more species containing B chromosomes, *viz. C. pannonica* and *C. conyzaefolia*. As already reported earlier (BABCOCK and JENKINS, 1943) the nor-





Figs. 1—2. The accessory chromosomes of *Crepis pannonica*. — Fig. 1. Somatic metaphase from a plant which in the root tips had 2n=8+1 acc. chr. — Fig. 2. Metaphase I showing 2 accessories forming a bivalent. In the root tips only one acc. chr. was found. — Fig. 1 c:a 700× and Fig. 2 c:a 350×.

mal chromosome number in these two species is 2n=8. We found, however, that certain individuals of these species contain a number of accessory chromosomes besides their 8 normal chromosomes (A picture of first metaphase in *Crepis pannonica* showing two accessories was exhibited by us at the Xth International Congress of Genetics, Montreal, 1958 in the exhibition arranged by the Institute of Genetics, Lund, and the Swedish Seed Association, Svalöf.).

Our plants of *Crepis pannonica* were grown from seeds obtained from the Botanical Garden of the Hungarian Academy of Sciences, Vácrátót. Five plants were studied and, when their root tips were investigated, three of them showed only the normal eight chromosomes, while two plants in addition to these had one accessory chromosome. The length of the accessory chromosome is about 1/3 of the smallest chromosome of the normal complement and it has a subterminal centromere (Fig. 1). The accessory chromosomes of this material have not shown any evidence of heteropycnosis, for which reason they must be considered as euchromatic, this expression being used in its original sense as a morphological concept.

Good fixations of meiosis in the pollen mother cells were obtained from one of the B-chromosome plants of *C. pannonica*. Although this plant had a single accessory chromosome, only, in the root tips, there were found two of them in the pollen mother cells. These two accessories were found to pair very regularly with one another to form a small bivalent (Fig. 2). The bivalent shows normal behaviour at first

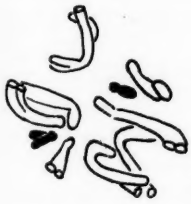


Fig. 3. Somatic metaphase from a plant of *Crepis conyzaefolia* with 2n=8+2 acc. chr. — C:a 1000×.

metaphase and chromosome separation at anaphase is also normal. No pairing was found between the accessory chromosomes and the chromosomes of the normal complement. In this respect these chromosomes behave in the same way as is characteristic of accessory chromosomes in general. Unfortunately, our data are not sufficient to ascertain whether the same difference in the number of accessory chromosomes between root tips and pollen mother cells exists also in the other plant, that also had a single accessory chromosome in its root tips. The observed difference between root tips and anthers is interesting but it would be premature to generalize from these observations on a single plant.

The accessory chromosomes of *Crepis conyzaefolia* have, as yet, been studied in root tips only. Our plants were grown from seeds obtained from two different sources, *viz.* from Botanischer Garten der Universität, Zürich, Switzerland, that had collected the seeds at Zernez, Lower Engadine, Switzerland, and from Parco Nazionale Gran Paradiso, Giardino Alpino a Valnontey, Cogne (Aosta), Italy, that also sent seed material collected in nature. (The Italian material of the species belonged to the variety *grandiflora*.) Accessory chromosomes were found in plants raised from seeds from both these sources and in altogether 27 plants the following chromosome numbers were found: 2n=8 in fifteen plants, 2n=8+1 acc. chr. in five plants, 2n=8+2 acc. chr. in five plants and 2n=8+3 acc. chr. in two plants.

The accessories in *Crepis conyzaefolia* (Fig. 3) agree in their morphology with those found in *Crepis pannonica*. They are small with a sub-terminal position of the centromere and they are euchromatic.

The evidence for the opinion that these chromosomes are accessories is as follows: In *C. pannonica* they do not pair with the chromosomes of the normal complement although they show a good pairing with one another. In *C. conyzaefolia* supernumerary chromosomes of the same morphological type were found in material from two separate populations and a number of plants contained more than one of them. Chromo-

some fragments derived by simple structural changes from the normal complement are not likely to occur in this way.

The morphological similarity of the accessories between the two different species studied by us suggests that it may be the same type of accessory chromosome or, at least, more or less related types in both of these species. Although the two species belong to different sections of the genus, it is possible to produce hybrids between them (BABCOCK, 1947 a, p. 59). The accessory chromosomes of *Crepis syriaca*, on the other hand, represent another morphological type. They are satellited chromosomes (CAMERON, 1934).

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DISTRIBUTION AND INHERITANCE OF MID-DIGITAL HAIR IN SWEDEN[1]

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THE first publication on the genetics of mid-digital hair by DANFORTH (1921) presents family data suggesting that complete absence of hair is due to a single recessive gene difference. Later BERNSTEIN and BURKS (1942) went into more detail by studying the family pattern of different types of combinations of absence and presence of hairs on the mid-phalanges. BERNSTEIN suggested a hypothesis of five different alleles, A_0, A_1, A_2, A_3 and A_4, to account for her family data.

BOYD (1950) studied the frequency of individuals with and without mid-digital hair in different populations. BERNSTEIN (1949) studied the frequencies according to the above mentioned allele system in an European sample of 200 individuals. MATSUNAGA (1956) found that the frequency of individuals with mid-digital hair was 40 per cent in the Japanese and 70 per cent in the Germans. Furthermore he has shown that hair on the mid-phalanges is present in children at the age of two months.

This investigation is included in a series of critical studies on traits that are used as evidence in cases of disputed paternity in some countries, including Sweden. The material consists of a sample of one hundred Swedish families selected by having one child at the approximate age when the law-suits commonly occur. All children were registered at Childrens Welfare Clinics in Uppsala. Their age varied between 1.5 and 4.5 years with a mean of 3.1 years.

The frequencies of hair on different digits for fathers, mothers and children showed no significant bimanual or sex differences (*cf.* Table 1). For the fifth finger ($P < 0.001$) and the fourth finger ($0.05 > P > 0.02$) the differences between parents and children were significant. This age difference was stronger on the ulnar side. MATSUNAGA (1956) and other workers did not find any differences by age, probably because

[1] aided by a grant from the Swedish Medical Research Council.

TABLE 1. *Presence of mid-digital hair on fingers 2, 3, 4 and 5.*

	n		Finger			
			2	3	4	5
Fathers	100	Right	6	46	61	31
		Left	6	50	64	33
Mothers	100	Right	6	41	64	34
		Left	7	49	62	33
Children	100	Right	10	48	54	17
		Left	9	45	52	17

they did not study the frequency variations for each finger. Furthermore as the children in our material were very young, age differences were easier to detect.

Table 2 shows the frequencies of presence and absence of hair among fathers, mothers and children (individuals lacking hair on both hands were classified as "absence"). The frequency among adult Swedes (71 per cent) shows a very good agreement with the frequency among Germans — 69.8 per cent (MATSUNAGA, 1956) and North-Americans — 70.4 per cent (DANFORTH, 1921). The frequency among children is slightly lower in our material. The difference is not significant, but from our knowledge of the age variations concerning the ulnar digits we may consider the difference real (if the development of hair on some fingers is dependent on age, children should be expected to show a lower frequency of hair totally).

The distribution of different digital combinations has been studied (Table 3 and Table 4). A regional subdivision of the material has also been carried out. Individuals born outside Sweden have been considered separately. BERNSTEIN (1949) claimed that different regions in Europe had typical combinations. Type A_s (hair on fingers 3, 4 and 5) should dominate in south Sweden and type A_0 (absence of hair) in north Sweden. The possibility that the occurrence of type A_0 in north Sweden

TABLE 2. *Frequencies of mid-digital hair.*

	+	−	n
Fathers	70	30	100
Mothers	72	28	100
Children	65	35	100

TABLE 3. *Distributions of mid-digital hair combinations (right hand).*

	Absence	Hair on fingers*										n
		3, 4, 5	4	3, 4	2, 3, 4, 5	2, 3	4, 5	3, 5	5	3	2	
North Sweden	14	10	5	6	2		4			1		42
%	33.3	23.8	11.9	14.3	4.8		9.5			2.4		100
Central Sweden	33	20	14	14	5		3	1	1	1		92
%	35.9	21.7	15.2	15.2	5.4		3.3	1.1	1.1	1.1		100
South Sweden	21	10	13	9	0	1	2					56
%	37.5	17.8	23.2	16.1		1.8	3.6					100
Born outside Sweden	1	2	1	0	4		1			1		10
%	10.0	20.0	10.0		40.0		10.0			10.0		100
Total	69	42	33	29	11	1	10	1	1	3		200
%	34.5	21.0	16.5	14.5	5.5	0.5	5.0	0.5	0.5	1.5		

* 2, 3—4, 5—3, 5—5—3 and 2 are so called atypical combinations.

TABLE 4. *Distributions of mid-digital hair combinations (left hand).*

	Absence	Hair on fingers										n
		3, 4, 5	4	3, 4	2, 3, 4, 5	2, 3	4, 5	3, 5	5	3	2	
North Sweden	15	12	4	6	2		2			1		42
%	35.7	28.5	9.5	14.3	4.8		4.8			2.4		100
Central Sweden	32	21	16	15	5				1	2		92
%	34.8	22.8	17.4	16.3	5.4				1.1	2.2		100
South Sweden	17	13	9	11	1		1	1	1	1	1	56
%	30.3	23.2	16.1	19.6	1.8		1.8	1.8	1.8	1.8	1.8	100
Born outside Sweden	1	2	1	1	4					1		10
%	10.0	20.0	10.0	10.0	40.0					10.0		100
Total	65	48	30	33	12	0	3	1	2	5	1	200
%	32.5	24.0	15.0	16.5	6.0	0.0	1.5	0.5	1.0	2.5	0.5	

TABLE 5. *Family combinations.*

Father	Mother	Children		n
		+	−	
+	+	38	12	50
+	−	12	8	20
−	+	14	8	22
−	−	1	7	8
	Total	65	35	100

+ = presence of mid-digital hair.
− = absence of mid-digital hair.

could depend on Lappish influence was also mentioned. The present material, however, showed no significant regional variations. The A_3-type had a frequency of about 23 per cent and the A_0-type of about 33 per cent (*cf.* Tables 3 and 4).

The frequency of atypical combinations was 7 per cent (*cf.* Table 3). BERNSTEIN and BURKS (1942) found 5 per cent and MATSUNAGA (1956) 3 per cent. Of 21 individuals (6 □ : 15 ○) with atypical combinations on one or both hands in this material 18 displayed asymmetry with respect to the right and left hand. Three individuals were symmetrical for the atypical combination 4, 5.

Family combinations with respect to presence or absence of digital hair are shown in Table 5. The results are consistent with the hypothesis of DANFORTH (1921) insofar as presence of mid-digital hair shows dominance.

Among the offspring in families where both parents possess mid-digital hair there was a significant deficiency of individuals with mid-digital hair ($P < 0.001$). The occurrence of one child with mid-digital hair in a family with both parents lacking the trait indicates that mid-digital hair is not a simple mendelian character. MATSUNAGA (1956) reports eight such "exceptions".

The results of this investigation show that the trait "mid-digital hair" cannot be used to exclude false paternity. However, it could possibly have some value if a very large family material was collected from which empirical probabilities could be calculated. Until such data have become available the trait is useless in forensic connections.

SUMMARY

(1) The frequency of the trait "mid-digital hair" in Sweden is 71 per cent which agrees with data from the United States and Germany. No geographical variation was found.

(2) The data from 100 families are consistent with DANFORTH's hypothesis insofar as the presence of mid-digital hair shows dominance. A significant age difference concerning the presence of mid-digital hair on the ulnar digits was found. The observations cannot be explained in terms of a simple Mendelian inheritance.

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STOCK DIFFERENCES IN X-RAY MUTATIONAL SENSITIVITY PATTERN OF DROSOPHILA MELANOGASTER

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I. INTRODUCTION

THE frequencies of spontaneous X-chromosome lethals in fifteen wild type stocks of *Drosophila melanogaster* were published by DEMEREC in 1937. He found that three of the stocks had a significantly higher mutation rate than the other twelve stocks. In another experiment DEMEREC (1938 a) investigated the X-ray induced mutation rate of X-chromosome lethals. For the same dosage, the mutation rate in the Swedish-b stock is about twice as high as in the Oregon-R stock. When the same two stocks were tested for X-ray induced dominant lethals then Oregon-R exhibited the higher frequency (DEMEREC, 1938 b). In other words the experiments show that there may be significant differences as to spontaneous and induced dominant and sex-linked lethal rates between wild type stocks. Furthermore, if one stock is higher with respect to one of these mutation rates, it does not necessarily follow that it also is higher with regard to the other types of mutation rate.

Since we were interested in finding a stock with low natural and induced mutation rate, and preferably also in requiring a long time for development, several stocks were investigated. The results obtained for two of these will be presented here.

Due consideration is given factors which influence mutation rate, such as age of the male at time of irradiation (DEMPSTER, 1941) and the brood used for scoring the mutation rate (LÜNING, 1952).

II. MATERIALS AND METHODS

The stocks employed were Iso-Amherst and Oslo. The Iso-Amherst stock was obtained ten years ago from Dr. C. STERN, Department of Zoology, University of California. The stock is originally a Canton stock made isogenetic several years ago with the fourth chromosome from Amherst. The other stock, Oslo, was collected outside Oslo, Norway, about twelve years ago, by Dr. VILHELM KIIL.

Males 1—4 hours old were treated with 1800 r of X-rays and mated in single pairs to virgin females. The males were transferred to a new vial and provided with a new virgin female every 24 hours for 12 days. This procedure was adhered to in all tests if not otherwise indicated. The stocks were tested with regard to dominant lethals, sex-linked lethals, and translocations between chromosomes 2 and 3. The frequency of dominant lethals is scored as the frequency of non-hatching eggs. The egg-laying females were virgin daughters from a cross between *cn bw*; e^{11} ♀♀ and Canton S ♂♂. As controls, untreated males 1—4 hours old were used.

Sex-linked lethals and 2—3 translocations were scored only in the third, sixth and eighth brood. All other broods were discarded. The frequency of sex-linked lethals were determined by the Muller-5 technique. F_2 cultures containing no wild type males were carried through F_3 and sometimes F_4, by transfer of five heterozygote females to a new culture bottle. If the irradiated X-chromosomes were carried through at least two generations with more than 25 M5 ♂ and no wild type ♂ in each, it was scored as a lethal.

Tests were also carried out for lethal clusters in the sixth and eighth broods.

The 2—3 translocations were scored in tests employing a *cn bw*; e^{11} stock.

All cultures were raised at a temperature of 24° C ± 1.5° C.

III. RESULTS

Dominant lethals

The 1—4 hours old treated males were mated individually to one new virgin hybrid female every 24 hours for 12 days. The hybrid females were 3—4 days old. The eggs laid were stored for at least 30 hours, and then the number of hatched and unhatched eggs were recorded. The same procedure was adopted for a control series with untreated males. The data are presented in Table 1 and in Figure 1. From these it can be seen that for each of the seven first broods the average hatchability is higher in the Iso-Amherst than in the Oslo stock. In the last five broods, however, the situation has changed, so the broods from the Oslo stock exhibit the higher hatchability. It seems that the sterile period clearly comes earlier in the Oslo stock and lasts for a shorter time than in the Iso-Amherst stock.

The control series shows that the Oslo stock possesses higher hatch-

TABLE 1. *Hatchability in broods 1—12 for the Iso-Amherst and the Oslo stock.*

	Control series				Treated series			
	Iso-Amherst		Oslo		Iso-Amherst		Oslo	
Brood	E	% H	E	% H	E	% H	E	% H
1	2002	97.00	2139	98.41	758	72.96	1004	65.04
2	3761	97.29	3201	97.72	2713	76.48	2233	72.77
3	1985	97.13	2146	97.67	2213	75.96	1646	71.14
4	2207	96.87	2378	97.10	1710	61.64	1140	53.95
5	3943	97.82	3314	98.46	1649	32.44	2447	25.95
6	2379	98.28	1612	97.64	2476	24.07	2060	15.34
7	1457	96.16	1706	98.77	645	20.16	699	13.45
8	2199	97.04	2296	98.71	1172	12.12	527	19.17
9	2541	97.17	2227	98.02	355	17.75	468	31.84
10	2276	98.64	1781	98.15	1098	18.58	706	67.71
11	1316	98.33	1294	98.38	1123	37.40	1029	81.44
12	2246	98.35	2642	98.79	592	69.43	916	91.48

The males in the treated series were given 1800 r of X-rays when the males were 1—4 hours old.

E=Total no. of eggs. %H=percentage hatched eggs.

ability than the Iso-Amherst stock. Neither of the two controls exhibits a brood pattern like that found for the same stocks after irradiation.

The overall hatchability for the 12 broods in the Iso-Amherst stock is 97.51 % and in the Oslo stock 98.15 %.

Translocations

Only the third, sixth and eighth broods were tested for 2—3 translocations. The results are given in Table 2. As can be seen from the table the highest frequency of translocations occurs in brood eight in the Iso-Amherst stock while it occurs in the sixth brood in the Oslo stock.

TABLE 2. *Translocations in brood 3, 6 and 8 after treatment with 1800 r of X-rays of 1—4 hours old males from Iso-Amherst and Oslo stocks.*

	Iso-Amherst			Oslo		
Brood	N	T (2—3)	% T (2—3)	N	T (2—3)	% T (2—3)
3	532	14	2.63	600	16	2.67
6	160	5	3.13	123	9	7.32
8	79	4	5.06	91	5	5.49

The table gives the total no. of sperms tested, the no. of 2—3 translocations and their frequency.

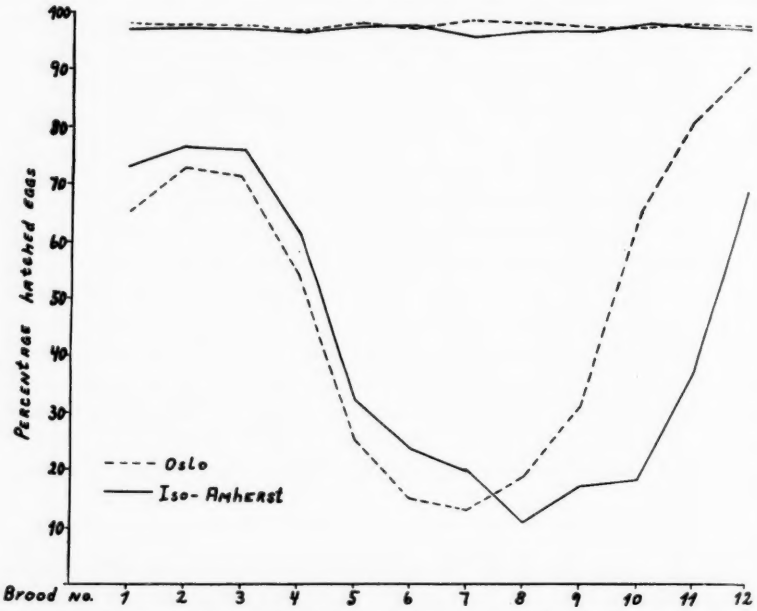

Fig. 1. The figure pictures the hatchability in broods 1—12 for the Iso-Amherst and the Oslo stock. The males in the treated series were given 1800 r of X-rays when the males were 1—4 hours old.

This would indicate that in brood 8 in Oslo stock meiotic elimination is already starting, while it probably has not taken place in the Iso-Amherst stock for the same brood. Although the stocks differed from each other in regard to the most sensitive broods, within each stock the highest frequencies of dominant lethals and translocations occurred in corresponding broods.

Sex-linked lethals

Table 3 lists the frequency of induced sex-linked lethals in brood 3, 6 and 8 from males treated 1—4 hours old. It can be seen that in the Iso-Amherst stock the mutation frequency increases and is largest in brood 8, while in the Oslo stock the frequency in brood 6 and 8 is almost the same. This may be taken as indicating the same pattern as shown for dominant lethals.

The data presented all indicate that there is a different sensibility

TABLE 3. *X-chromosome lethals in brood 3, 6 and 8 after treatment of 1—4 hours old males with 1800 r of X-rays.*

	Iso-Amherst			Oslo		
Brood	N	1	% 1	N	1	% 1
3	427	9	2.11	1473	48	3.26
6	405	16	3.95	478	26	5.44
8	228	12	5.26	520	26	5.00

N=number of X-chromosomes tested.
1=number of X-chromosome lethals.

pattern of the two stocks. Thus, the peak value for mutations induced comes in an earlier brood in the Oslo stock than in Iso-Amherst stock. This difference may be explained if we suppose an earlier production of sperms or earlier appearance of sexual activity in one stock than in the other. Beside this difference between stocks, another is probably expressed in the prolongation of the sterile period in the Iso-Amherst stock.

To test the rates of development in the two stocks, eggs were collected during a four hour period and transferred to vials for developing. Hatching flies were counted and the average time required for development calculated. The data indicate a slightly longer developing time for the Iso-Amherst than the Oslo stock when raised under approximately optimum conditions, that is at a temperature of 25° C and with extra yeast suspension added to the medium during larval development. The difference in developing time was about five hours. If they developed at 22° C the difference in developing time was about 42 hours. Oslo required 11 days 12 hours for development and Iso-Amherst 13 days 6 hours.

Mutation rate in mature sperms

Three day old males from both stocks were treated with 1800 r of X-rays and only the sperms utilized during the first 24 hours after treatment were tested for sex-linked lethals. Unirradiated 3 day old males from the same stocks were used as control.

The results given in Table 4 indicate that the spontaneous mutation rate as well as the X-ray induced mutation rate is higher in the Iso-Amherst than in the Oslo stock. This was not expected. In all tests with 1—4 hours old males the Iso-Amherst stock exhibited the lower mutation rate, but when 3 day old males were used this stock exhibits the higher

TABLE 4. *Spontaneous and induced X-chromosome lethals in first brood.*

	Iso-Amherst			Oslo		
Treatment	N	1	% 1	N	1	% 1
Control	6216	10	0.16	6816	5	0.07
Treated	2537	111	4.38	1984	72	3.63

The males were 3 days old when treated with 1800 r of X-rays.

mutation rate. However, the latter observation is in agreement with older work on mature sperms from the same two stocks. Table 5 summarizes the results found for the two stocks. It seems obvious that here is revealed a between-stocks difference in sensitivity of sperms from younger and older males that previously has escaped notice.

IV. DISCUSSION

The purpose of this experiment was to establish the brood pattern for a stock to be selected for later radioactive isotope studies. Therefore a search was made for a slow developing stock with low natural and induced mutation rates. The hatchability brood pattern for eggs from females inseminated by irradiated males was similar to that already shown by other authors (LÜNING, 1952; AUERBACH, 1954; KHISHIN, 1955 and BATEMAN, 1956).

In all experiments only one female was placed with a given male at

TABLE 5. *Relative frequency of induced mutations in the Iso-Amherst and the Oslo stock for the broods coming the first six days after treatment, when the males are 1—4 hours old, and when they are 3—4 days old at the time of treatment.*

	Iso-Amherst	Oslo
1 to 3-hour-old males:		
Dominant lethals, control	Low	High
Dominant lethals, 1800 r	Low	High
Translocation, 1800 r	Low	High
Sex-linked lethals, 1800 r	Low	High
3 to 4 day-old males:		
Sex-linked lethals, control	High	Low
Sex-linked lethals, 1800 r	High	Low
Dominant lethals, 2300 r (STRØMNÆS, 1951)	High	Low
Dominant lethals, 2300 r (STRØMNÆS, 1955)	High	Low

one time, thus, assuring an adequate supply of sperm for the fertilization of all eggs. This should avoid the difficulty demonstrated by MOSSIGE (1955) who showed that eggs fertilized by sperms from males mated to their utmost capacity have a low hatchability. In each of the two stocks reported on here, less than 2.5 % of eggs failed to hatch in the control series. Thus, the mating procedure and the moderate dosage of 1800 r render it likely that the majority of unhatched eggs in the sterile period represents dominant lethals induced in the sperms.

The data for sex-linked lethals and translocations support the picture of the sensitivity pattern given by the curves for the dominant lethals. It seems, therefore, justifiable to conclude from the evidence presented that there is a significant difference in mutation pattern between the two stocks. The more sensitive stages in the Iso-Amherst stock come in later broods than in the Oslo stock. To explain this difference between the two stocks the developing time from egg to imago must be taken into consideration. This time is shorter in the Oslo stock than in the Iso-Amherst stock. Thus, more mature sperms are present at time of irradiation in the 1—4 hours old males of the Oslo stock than in the other. Since the time required for eggs to develop into imagoes is greater in one stock than in the other, it seems likely that a similar difference would occur with respect to the time after eclosion at which the first motile sperms appear.

According to KHISHIN (1955, 1957) the first motile sperms in males of the Oregon-K stock were observed 7 ± 1 hours after eclosion. If the motile sperms first appear a few hours later, this may cause the apparent shift in mutation pattern between stocks.

A between-stock difference in mutation rate as observed here may be the cause of the discrepancy pointed out by Dr. AUERBACH (1954) between her own data and Dr. LÜNING's.

Another interesting difference between stocks is revealed in reviewing all the mutation rates found for the two stocks (Table 5). Here we find a strange between-stocks difference in the effect of radiation on sperms from 1—4 hour old males vs. 3—4 day old males. One hypothesis to explain this difference may be the presence of radiation protective chemicals in the sperm. The amount of such chemicals may differ in sperms from different stocks, and may also differ with the age of the male. Thus, LÜNING has shown (1958) that the spermatids in older males are less sensitive than spermatids in young males. That this should be due to mixing of sperms of different age in the older males as suggested by MOSSIGE was ruled out by LÜNING.

Partial support for the hypothesis suggested may be found in unpublished data by Dr. LÜNING (personal communication). He has investigated the mutation rates in sperms ready for fertilization at different times after irradiation. The mating periods were from one to three hours. He found that in sperms from males 0—1 day old when irradiated the mutation rate suddenly dropped in sperms used for fertilization about four hours after irradiation, as compared to those used earlier. When the same investigation was carried out on sperms from 3—4 day old males a corresponding drop in mutation rate was first observed in the sperm batch inseminated about 15 hours after irradiation.

Thus, there is a distinct difference in mutation pattern of mature sperms from young vs. older males. Protective chemicals may be responsible for this difference. Since the two stocks reported on here have different rates of development the metabolism going on in the sperm may also have different rates. This can then cause the change in relative mutation frequency between males of the two age groups of the two stocks.

None of the data presented here makes it possible to tell whether the suggested protective chemicals act on the breakage process or the rejoining process.

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SUMMARY

The mutation pattern of two wild type stocks of *Drosophila melanogaster*, Iso-Amherst and Oslo, has been investigated. The data for egg hatchability, sex-linked lethals, and 2—3 translocations all indicate that sperms available for fertilization the six first days after irradiation of young males of the Oslo stock have a higher mutation frequency than do the sperms from males of the Iso-Amherst stock. Furthermore, the brood with the highest mutation frequency comes earlier in the Oslo stock than in the Iso-Amherst stock. This may be ascribed to differential maturation rates in the two stocks.

When older males of the same two stocks are irradiated then the mutation frequency in the broods coming the first six days after irradiation indicate that the Iso-Amherst stock is the more sensitive one.

The change in sensitivity to X-ray induced mutations may be ascribed to different metabolic rates in the two stocks.

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CHROMOSOME DISTURBANCES AND MUTATION PRODUCED IN PLANT SEEDS BY OXYGEN AT HIGH PRESSURES

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IN a preliminary investigation (EHRENBERG, J. and M. MOUTSCHEN-DAHMEN, 1957) a treatment of barley seeds with high pressure oxygen was found to induce appreciable frequencies of chromosome aberrations. In order to permit a more detailed investigation of the effect the present investigation was started, comprising a similar treatment of seeds of *Vicia faba*, *i.e.*, a more suitable material for cytological studies. It was further studied, in barley, whether O_2 treatment induces gene mutation.

MATERIAL AND METHODS

In order to make the gas : seed volume ratio as large as possible in the steel containers used for the treatment, the small-seeded variety *minor* of *Vicia faba* (Weibull's "Åkerböna") was chosen for the investigation. Seed samples were treated, at 20—22° C., for 3 days to 4 weeks at oxygen pressures amounting to 30 or 60 atmospheres. As controls were used an untreated sample and, in addition, seeds treated at 60 atm. of nitrogen for different time periods.

In order to avoid the reversible (*cf.* EHRENBERG *et al.*, 1957) appearance of primary lesions, which disturb the counting of fragments, the germination of the seeds was not started until 3 to 4 weeks after the end

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of the gas treatment. The seeds were germinated in petri dishes on moist filter paper.

In the present study, we were interested in determining the changes in the frequencies of induced lesions during the first three nuclear divisions in the root meristems after the onset of germination. It was therefore necessary to identify the mitotic succession up to the third division. For this purpose, roots were fixed at intervals of 20 to 40 hours, 70 to 80 hours, and 90 to 125 hours after the sowing of the seeds on filter paper. We have identified that at the germination temperature, 20—22° C., these periods correspond very regularly to the first three mitoses. — According to HOWARD and PELC (1953), 30 hours are required for the completion of one mitotic cycle in growing roots of the same material. As has already been pointed out in earlier investigations concerning this question (J. and M. MOUTSCHEN-DAHMEN, 1958 a, b), the appearance of prophasic micro-nuclei has been used, classically, as a means to identify the start of the second mitosis.

Fixation was done in ethanol+acetic acid (3 : 1) or in Carnoy's mixture, and the nuclear material was stained, in squash preparations, according to the Feulgen method.

For a determination of induced gene mutation, samples of *barley* seeds were treated for 7 to 56 days at an oxygen pressure of 50 atmospheres. The material was sown in the field in the spring of 1958. In the autumn, 1958, the second generation was analysed in a green-house test, according to the methods worked out by GUSTAFSSON (1940), with respect to the frequencies of spike progenies segregating for chlorophyll deficient mutants.

TABLE 1. *Frequencies, per metaphase, of isoloci chromosome breaks identified in metaphase.*

(Each number is based on about 500 metaphases)

Treatments	Mitosis	Duration of treatment in days				
		3	7	15	22	28
O_2 30 atm.	1st	0.06	0.10	0.15	0.25	0.23
	2nd	0.12	0.22	0.27	0.72	0.18
	3rd	0.075	0.166	0.174	0.20	0.20
O_2 60 atm.	1st	0.06	0.10	0.15	0.17	0.22
	2nd	0.19	0.21	0.47	0.76	1.20
	3rd	0.10	0.12	0.19	0.20	0.21
N_2 60 atm.	2nd	0.001	0.01	0.01	0.02	0.042

EXPERIMENTAL RESULTS

The effects of oxygen on the frequencies of chromosome breaks in metaphase and anaphase and on the frequencies of anaphase bridges have been studied. The observed frequencies at metaphase are summarized in Table 1. In this table, we have only regarded the frequencies of "isoloci" breaks, excluding the other observed breaks; the low frequencies of the latter are practically without any influence on the total frequencies.

Throughout, the controls exhibited insignificant frequencies of disturbed cells, not included in the tables. From Table 1 it is seen that the frequency of breaks increases with the duration of oxygen treatment. The effect of nitrogen at the same pressure is very low in comparison with that of oxygen, but the low effect of nitrogen is nevertheless significant and also shows an increase with the duration of treatment. Regularly, the highest aberration rates are observed at the second mitosis after the gas treatment. It is also at this division that the increase of the aberration rate with dose is greatest. At the first mitosis, this increase is significant only at the lowest doses (shortest treatments).

Going from the second to the third mitosis, a sharp fall in the frequency of breaks is observed. This indicates a rapid elimination of the oxygen induced lesions, similar to that observed for the effects of ionizing radiations and the strongest nucleotoxic chemicals, *e.g.*, ethyleneimine.

Comparing the effect of the two pressures used, 30 and 60 atmospheres, it is evident that the difference between the samples treated for the same length of time is rather small and is significant only for the treatments of short duration.

TABLE 2. *Frequencies of chromosome breaks identified in anaphase.*
(Each number is based on about 500 anaphases)

Treatments	Mitosis	Duration of treatment in days				
		3	7	15	22	28
O_2 30 atm.	1st	0.030	0.09	0.100	0.110	0.14
	2nd	0.080	0.13	0.320	0.835	1.445
	3rd	0.015	0.07	0.110	0.230	0.29
O_2 60 atm.	1st	0.16	0.171	0.196	0.220	0.246
	2nd	0.39	0.410	0.886	1.275	1.469
	3rd	0.086	0.10	0.125	0.295	0.350
N_2 60 atm.	2nd	0.002	0.01	0.006	0.008	0.020

TABLE 3. *Frequencies of anaphase bridges per anaphase.*

(Each number is based on about 500 anaphases)

Treatments	Mitosis	Duration of treatment in days				
		3	7	15	22	28
O_2	1st	0.020	0.04	0.08	0.10	0.16
30 atm.	2nd	0.042	0.074	0.21	0.50	0.82
	3rd	0.025	0.046	0.076	0.21	0.22
O_2	1st	0.08	0.095	0.11	0.15	0.18
60 atm.	2nd	0.127	0.255	0.46	0.538	0.86
	3rd	0.04	0.08	0.15	0.17	0.23
N_2	2nd	0.002	0.015	0.016	0.016	0.020
60 atm.						

Table 2 summarizes the data concerning the frequencies of chromosome breaks at anaphase. Making comparisons with the metaphase data, the following can be stated:

(1) nitrogen produces a number of breaks corresponding to those observed at metaphase; here also an increase with the duration of treatment is evident. Observations at this stage confirm the data from metaphase, demonstrating that the effects of nitrogen are very small compared to those of oxygen.

(2) the highest frequencies of anaphase fragments are determined at the second mitosis, as was the case for the chromosome breaks at metaphase.

(3) in similarity with the observation at metaphase, the elimination of anaphase fragments is very rapid. On an average, 75 per cent of the anaphase fragments observed in the second division cannot be recovered in the following mitosis.

TABLE 4. *Percentages of disturbed metaphases.*

(Each number is based on about 500 metaphases)

Treatments	Mitosis	Duration of treatment in days				
		3	7	15	22	28
O_2	1st	6.3	9.1	10	22.1	24.4
30 atm.	2nd	12.6	16.9	20.2	54.5	76.6
	3rd	11.6	12.1	14.7	17.8	28.9
O_2	1st	6.5	13.3	19.4	*	*
60 atm.	2nd	22.9	30.1	46.6	56.2	99.6
	3rd	17.2	16.8	22.3	27.8	30.5
N_2	2nd	0.5	1.1	1.6	3	3.3
60 atm.						

* Observations impossible due to disturbing influence of primary effects.

(4) the difference between the two pressures is more evident than was demonstrated in Table 1, but is nevertheless restricted to the treatments of shorter duration.

The rejoining ability of broken chromosomes is estimated by an evaluation of the frequency of anaphase bridges observable at the first three mitoses. In Table 3, where these data are summarized, it is demonstrated that the frequencies of bridges run parallel to, but are often somewhat lower than those of the fragments in the same treatment. Many of the fragments are acentric and may therefore be eliminated, or recovered as micronuclei in the following prophase. The conclusions drawn from Tables 1 and 2 concerning the effects of pressure and duration of treatment are valid also for the frequencies of bridges.

In the Tables 4 and 5, the effect of oxygen is given, summarily, as the percentages of disturbed mitotic figures at metaphase and anaphase, respectively. As disturbed mitotic figures, we understand cells which contain one or several lesions of the following types:

(1) chromosomal fragments
(2) chromosomal micro-fragments or minutes
(3) micronuclei
(4) identifyable chromosome translocations
(5) identified deficiencies and deletions
(6) tri- or quadri-radial configurations.

These tables thus give a general view of the extent of action of oxygen, without entering into analytical details as was done in the Tables 2—3. (A more detailed qualitative description of the lesion types observed is going to be given in a later communication by M. and J. MOUTSCHEN-DAHMEN, 1958 c.)

TABLE 5. *Percentages of disturbed anaphases.*

(Each number is based on about 500 anaphases)

Treatments	Mitosis	Duration of treatment in days				
		3	7	15	22	28
O_2 30 atm.	1st	4.3	10.2	11.1	11.5	12.4
	2nd	12.7	15.6	22.3	65	84.1
	3rd	4.1	9.5	16.1	21.2	22.1
O_2 60 atm.	1st	11.6	12.6	15.6	23.9	31.1
	2nd	23.3	31.5	57	81.2	98.6
	3rd	9.1	15.9	15.9	23.4	36.6
N_2 60 atm.	2nd	0.5	1	2	2.5	4

TABLE 6. *Frequencies per metaphase of chromosome reunions.*
(Each number is based on about 500 metaphases)

Treatments	Mitosis	Duration of treatment in days				
		3	7	15	22	28
O_2 30 atm.	2nd	0.010	0.015	0.032	0.125	0.138
O_2 60 atm.	2nd	0.050	0.063	0.121	0.118	0.127
N_2 60 atm.	2nd	0	0	0	0	0

The rapid fall of the frequency of disturbed mitotic configurations after the second division is more pronounced at higher doses, *i.e.*, longer treatments. This fact, which is evident especially at the metaphase stage, might be of importance for interpreting the mode of action of oxygen. The relationships between oxygen pressure, duration of treatment, and the frequencies of different disturbances determined at different stages of the first three mitoses are rather complicated, and it is therefore not excluded that more than one mechanism of oxygen action is at work. The more rapid decrease between the second and third divisions of the frequency of disturbed cells at higher doses could therefore be explained by a slower action of oxygen in the case of short treatments, although no evidence, from a chemical point of view, can at present be given in favour of such an interpretation. It seems more plausible that the difference in frequencies of disturbed cells has a bearing on reunion rather than breakage occurring at different rates after short and long treatments, respectively. If there were proportionally more reunions after short oxygen treatments, this would indicate an inhibition of reunion and, consequently, a more rapid elimination at higher dosages.

In Table 6, the frequencies of chromosome reunions at the metaphase of the second mitosis are given. As reunions, we have considered here all forms of chromosome translocations as well as tri- and quadri-radial configurations. The table demonstrates an increase with dose of the number of reunions observed at metaphase, as was also found for the anaphase bridges (Table 3). An inhibition of reunions can therefore hardly be a main cause of the sudden decrease of disturbances in the third mitosis.

It seems more probable that the phenomenon is due to a dominating elimination of certain types of lesions, or even entire cells, at high doses. Investigating the question of which types of lesions could be principally

TABLE 7. *Frequencies of micro-fragments (minutes) and micronuclei.*

Treatment	Mitosis	Duration of treatment in days				
		3	7	15	22	28
O_2	2nd	0.04	0.052	0.15	0.38	0.825
60 atm. minutes	3rd	0	0	0.007	0.04	0.05
O_2	2nd	0.023	0.053	0.059	0.100	0.12
60 atm. mitotic micronuclei	3rd	0.005	0.009	0.011	0.02	0.03
N_2	2nd	0	0	0	0	0
60 atm. minutes and micronuclei	3rd	0	0	0	0	0

involved in the more marked elimination after treatments of long duration, we note that there is an appreciable increase with dose of the following lesions:

(1) chromosomal micro-fragments (minutes);
(2) micronuclei;
(3) cells containing, each, several aberrations.

The third type of disturbance can be lethal to the cell which is, therefore, by necessity rapidly eliminated by its inability to divide. The minutes and non-nucleolated micronuclei (which are, in principle, incapable of synchronized division) are labile, being easily resorbed by the cytoplasm in the course of a mitotic cycle. Table 7 indicates that especially the frequency of minutes strongly increases with dose, and that this type of lesion is also most effectively eliminated. At the highest dose, more than 90 per cent of the minutes observed in the second metaphase have disappeared in the third division. For the mitotic micronuclei, on an average, 80 per cent are simultaneously eliminated. Obviously, minute aberrations play a dominating role for the elimination from 2nd to 3rd divisions, demonstrated in Table 4.

Qualitative effects of oxygen. — To sum up, it can be stated that all types of lesions produced by ionizing radiations are recovered as a consequence of the action of oxygen at high pressures (*cf.* also CONGER and FAIRCHILD, 1952). Also, at the first mitosis after treatment, primary (or "physiological") modifications of the chromosomes are found, as has been preliminarily reported for barley (EHRENBERG, J. and M. MOUTSCHEN-DAHMEN, 1957). These effects are rather labile, and we have noted their reversibility. In the succession of events, they give place to chromosomal breaks and reunions. The majority of the breaks are

iso-chromatidic; only very few other breaks have been observed. — The treatment also gives rise to spindle abnormalities, leading to:

(1) mitoses with distorted spindle;
(2) mitoses with multipolar spindle;
(3) whole lagging chromosomes, with one or several centromeres;
(4) mitoses similar to "C-mitoses".

Localisation of breakage points. — Most nucleotoxic chemicals exhibit, compared to ionizing radiations, a more or less pronounced specificity regarding parts of the genome being preferentially damaged by the agent.

The oxygen effect has been investigated preliminarily from this point of view. A review of the localisation of 492 breakage points, studied at the second metaphase after treatment, demonstrated:

(1) no significant concentration of the breakage points to certain regions of the genome can be observed;

(2) the ratio $\frac{S}{M}$, *i.e.*,

$$\frac{\text{frequency of breaks produced in acrocentric chromosomes}}{\text{frequency of breaks produced in metacentric chromosomes}},$$

was found to be 2.49, in good agreement with the ratio of the lengths of the chromosomes in question, which was found to amount to 2.37 (*cf.* REVELL, 1953).

It follows, that the relative frequency of breaks in a given chromosome is proportional to the length of the chromosome. The random distribution of the oxygen induced chromosome breaks along the chromosomes is further visualized in Fig. 1, giving the exact localisation of 69 breakage points in the pair of metacentric chromosomes (M). This random distribution is closely similar to the effect of ionizing radiations (*cf.* REVELL's Fig. 1, 1953), and clearly different from the effects of chemicals investigated in this respect (*cf.* REVELL, *l.c.*; J. and M. MOUTSCHEN-DAHMEN, 1958 a, b).

Induced gene mutation. — Resting barley seeds treated under conditions similar to those used for the cytological studies of *Vicia* were found to lead to appreciable rates of what, in irradiation experiments, is generally regarded as gene mutations. The observations will be mentioned briefly in this connection, although the interpretation of the data will require an analysis also of the third generation. About the X_a spike

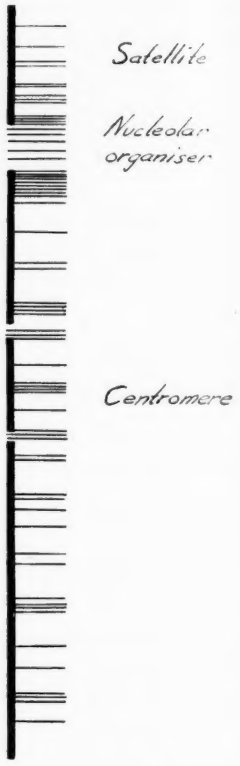

Fig. 1. Localisation of breakage points in the pair of metacentric (M) chromosomes.

progenies segregating for chlorophyll deficient seedlings, the following facts can be stated:

(1) As shown in Fig. 2, the dependence on dose (*i.e.*, duration of treatment) is clearly non-linear, no mutants at all being found after treatments for 7 or 17 days (*cf.* the 97.5 per cent confidence limits given in the graph). — From a chemical point of view this behaviour would indicate that the tissue normally contains a system protective against free oxygen (*cf.* EHRENBERG and EHRENBERG, 1958). When this system has become saturated with O_2, some oxidized species is found, which is toxic to the gene material (it could be noted that the curve is similar to that of hydroperoxide in autoxidizing fat). In the case of the longest treatment the toxic species is again destroyed by oxygen.

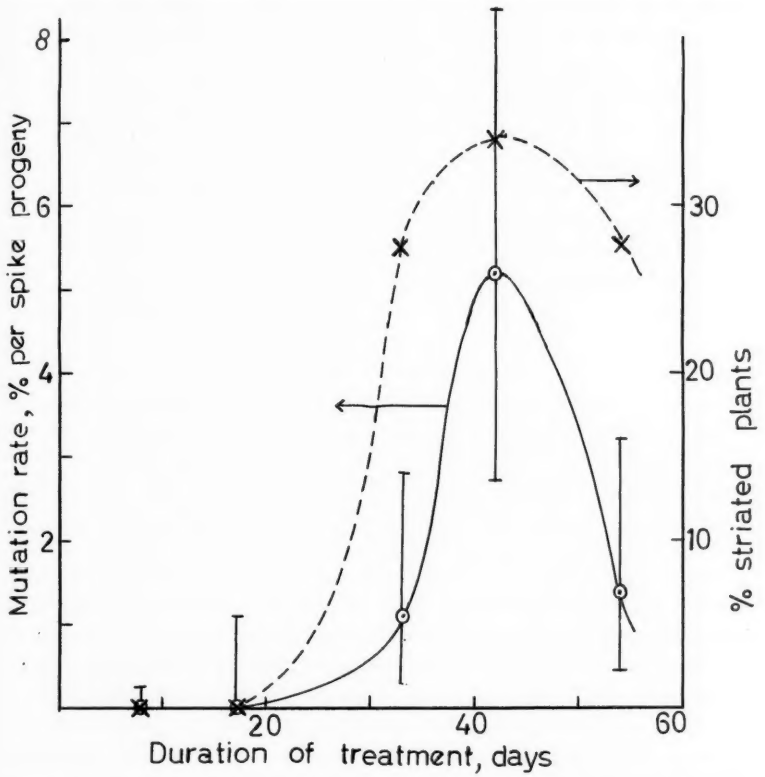

Fig. 2. Mutation rate per spike progeny (⊙), and frequencies of striated X_1 plants (×), as a function of duration of O_2 treatment.

It should be remarked that an explanation of this kind is still only tentative. In this preliminary study, all the treatments were started simultaneously, and, in consequence, the samples given the lowest O_2 doses were left longest under normal atmospheric conditions before sowing. As far as the effect is caused by a labile reactive principle, this would have the greatest chance of decaying in the last-mentioned samples.

(2) As regards the spectrum of mutations formed, oxygen exhibits a specificity different from those of all other mutagenic chemicals tested. Practically only *albina* mutants are found, *i.e.*, the mutant type most

TABLE 8. *Summary of the analysis of mutations induced in barley by treatment of resting seeds with 50 atmospheres of O_2.*

Duration of treatment, days	Number of spike progenies analysed	Number of mutated progenies			Mutation rate %
		albinae	others*	sum	
8	802	0	0	0	0.0
17	335	0	0	0	0.0
33	368	3	1	4	1.1
42	308	15	1	16	5.2
54	360	5	0	5	1.4
Sum of treatments		23	2	25	
control	521	0	4	4	0.8

Segregation ratios:

The sum segregation number (*i.e.*, total number of normal seedlings : total number of *albina* seedlings) equals 305 : 81, and is not significantly different from the 3 : 1 ratio (comparison with expected numbers, 289.5 : 96.5, gives $\chi^2=3.3$, 1 d.f., $0.10 > > P > 0.05$). This agreement is coincidental, however, since a test of the 3 : 1 segregation ratio within the individual spike progenies gives $\chi^2=32^{***}$ (11 d.f.), and a heterogeneity test gives, in agreement therewith, $\chi^2=42^{***}$ (10 d.f.) for a variation between progenies with respect to segregation ratio.

common in irradiation experiments (Table 8). When treatments with mutagenic chemicals lead to displacements of the spectrum in comparison with that obtained with X-rays or neutrons, the relative frequencies of *viridis* (EHRENBERG *et al.*, 1956; HESLOT, 1958) or rare types (EHRENBERG *et al.*, 1959) have been observed to increase. — In peas, oxygen has been found to induce different mutation types at fairly low rates (GELIN, EHRENBERG, and BLIXT, unpublished).

(3) Although the average segregation ratio is fairly close to 3 : 1, indicating a monofactorial inheritance, the individual spike progenies exhibit ratios significantly deviating from the average (*cf.* Table 8).

Discussing at this stage of the investigations the true nature of the mutants, the possibility cannot yet be excluded that chlorophyll-free sectors involving parts of the X_1 spike are directly transferred to the following generation. In the experiments with *Vicia faba* as well as barley, oxygen was found extremely effective in producing such sectors. In the *Vicia* series, parts of the seed samples were sown in the greenhouse for a counting of such light-coloured leaf spots, which are regularly produced, by irradiation and alkylating chemicals, in leguminous plants. The spots have been proved to be two-hit effects in the chromosome material, probably translocations (ZACHARIAS and EHREN-

BERG, unpublished; *cf.* KAPLAN, 1954). In the grass, barley, the "mutated" sectors form longitudinal chlorophyll-free streaks; such are rarely found after irradiation of seeds, especially with neutrons (*cf.* EHRENBERG and NYBOM, 1954). After oxygen treatment such streaks were very common, giving a striated impression to a great part of the X_1 plants. Although it was not clarified whether the occurrence of mutation in X_2 was correlated to a striation of the X_1 mother plant, the frequency of striated plants exhibits a dose dependence similar to that of the mutation rates. In X-ray experiments with red-clover, BRUNS (1954) found white sectors, nourished by the green parts of the plant, to give rise to white offspring.

DISCUSSION

Oxygen is a reactive compound, destroying most organic chemicals which are in contact with air for long times. The mechanism of action is at present not known in detail. Being a diradical, as is shown by its paramagnetic properties, oxygen can be adsorbed to electron-donating centers of different kinds. Such centers occur frequently in biologic materials, and the (reversible) adsorption of oxygen to these gives rise to magnetic centers detectable by means of electron spin resonance (MIYAGAWA *et al.*, 1958), probably similar to peroxy radicals, which might later form biologically active peroxides (such events form the probable mechanism, *e.g.*, in the formation of allergens from terpenes; *cf.* HELLERSTRÖM *et al.*, 1955). Such an interpretation of the present effects is not excluded by the fact that efforts to determine peroxides by traditional iodometric and ferrometric methods gave completely negative results. Irradiation of tissue leads to the production of large amounts of new electron-donating centers, free radicals, which combine rapidly with oxygen. Part of the radiation after-effects caused by oxygen, might be due to reactions of this type (*cf.* EHRENBERG and EHRENBERG, 1958).

From this point of view, it is of special interest that oxygen gives a distribution of chromosome breaks which is closely similar to that produced by X-rays but different from that obtained after treatment of seeds with different chemicals, including such reactive, expectedly unspecific agents as epoxides and peroxides (*cf.* REVELL, *l.c.*). This indicates a common or related mechanism of action of oxygen and ionizing radiations. The similarity between the effects of the two types of agent, in this investigation studied on the fine-structural level, adds

to other similarities, widely different in nature, obtained in different experimental objects (*cf.* GERSCHMAN *et al.*, 1954):

(1) equality of types of chromosomal disturbances (*cf.* CONGER and FAIRCHILD, 1952; J. and M. MOUTSCHEN-DAHMEN, 1958 c).

(2) production, by both agents, of gene mutation in *Escherichia coli* (FENN *et al.*, 1957) and also in higher plants, *Pisum sativum* (GELIN, EHRENBERG and BLIXT, unpubl.) and barley (the present investigation). Although the maximum mutation rates obtained with X-rays and O_2 are about the same, a difference seems to exist with regard to the spectrum of mutated types induced. With O_2, practically only *albinae* are found; with radiations this type constitutes about 40 per cent of the total mutation rate.

(3) both agents may lead to a break-down of DNA molecules (*cf.* GILBERT *et al.*, 1957).

(4) toxic changes produced by either agent in *Paramecium caudatum* similar (GERSCHMAN *et al.*, 1958).

(5) protective action of sulfhydryl compounds against oxygen poisoning as well as radiation damage (GERSCHMAN *et al.*, 1954).

One difference of major importance between the two agents might lie in the fact that after oxygen treatment, the highest frequencies of disturbances are found in the second mitosis after treatment, whereas in the case of X-irradiation, the maximum rate of disturbances is observed already in the first mitosis. On the other hand, the elimination of disturbances, after the reaction maximum, occurs at a corresponding rate in the two types of treatment. Such a delayed maximum is also shown for myleran (J. and M. MOUTSCHEN-DAHMEN, 1958 b) the action of which is specific, however, and might very well occur more slowly. (This is probably the case with other chemicals, too.) In the case of oxygen, *i.e.*, a small easily diffusing molecule, a more rapid action would be expected; its delayed effect may therefore possibly be ascribed to a slower reaction of some kind of intermediates.

The possible role of oxygen for the induction of spontaneous aberrations (and mutations) was discussed in our preliminary investigation, and also by EHRENBERG and EHRENBERG (1958). As a first step in the further study of this problem, it would be important to map the distribution within the genome of the sites of spontaneously occurring chromosome breaks.

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SUMMARY

(1) The chromosome breaking effect of O_2, applied at 30—60 atmospheres, was studied after treatment of seeds of *Vicia faba* for 3—28 days. High frequencies of fragments and anaphase bridges were induced. The effect is due to an action of O_2, since a similar treatment with N_2 at 60 atmospheres leads to the formation of aberrations at very low (although measurable) rates.

(2) The aberration rate is highest in the second mitosis after the onset of germination. It rapidly drops from 2nd to 3rd division; especially micronuclei and micro-fragments are eliminated rapidly.

(3) The breakage points were found to be distributed at random within the genome. In this respect the effect of oxygen is identical with that of X-rays but different from that of "radiomimetic" chemicals, which mostly attack specifically certain regions of the chromosomes (*e.g.*, heterochromatin or constrictions).

(4) Mutagenicity of high pressure O_2 was studied in barley, observing chlorophyll deficient seedlings in the second generation after treatment of seeds. Fairly high rates were found, chiefly of *albina* mutants. This completely chlorophyll-free type constitutes about 40 % of the mutants obtained with radiations, and less with most mutagenic chemicals. Thus oxygen gives a mutation spectrum not obtained with other agents studied. The true nature of these mutants is still somewhat obscure.

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A STUDY OF THE RETENTION AND THE MUTAGENIC MODE OF ACTION OF RADIOACTIVE PHOSPHORUS IN DROSOPHILA MELANOGASTER

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I. INTRODUCTION

RADIOISOTOPES are distinguished from stable isotopes by the fact that they emit radiation, and from this feature stems the characteristic of their uses in physics, chemistry and biology. The relative ease with which the emitted radiation may be registered quantitatively forms the basis for the use of radioisotopes as tracers, providing unequalled possibilities for detection of minute quantities. In these instances, the radiation itself is only the signal, indicating the position of the radioisotopes at the time, and this latter aspect is the one with which the investigator is concerned.

In other cases, the radiation emitted constitutes the main feature, its amount, quality or effects being foremost in the interest of the observer. This is so for the physicist investigating energy levels of the atomic nucleus, the chemist looking for radiation degradation of molecules, or the biologist measuring the relative biological effectiveness of different radiations.

Since radiation has a profound — and largely poorly understood — effect on living matter, it is sometimes difficult to keep these two features separate in radioisotope experiments involving biological material. Usually, tracer doses are kept so low that the radiation effects are negligible. This is so in most incorporation and turnover experiments. In some cases, however, it is necessary to use relatively high concentrations of radioisotopes in order to get a sufficient number of unstable atoms into the positions to be investigated, and in these cases the radiation effects on the test organism may influence the experimental results.

Radioisotope incorporation studies have been used by several authors

for the timing of spermatogenesis in the mouse as well as in larger animals (HOWARD and PELC 1950, ORTAVANT 1954 a, b, 1956, SIRLIN and EDWARDS 1955, PELC 1957, DAWSON 1958 a, b). Although cytologic studies may give a clear picture of the sequence of events, it is often so that tagging of sperm is a more reliable method when it comes to evaluation of the length of time occupied by the different stages. The cytologic timing of stages is difficult in *Drosophila*, and it was felt that incorporation data might give more reliable information than morphologic studies. The present investigation was therefore started primarily to measure the time needed for spermatogenesis by means of the tagging of sperm with P^{32}. It was thought that this would give information much needed in connection with the published genetic results showing the occurrence of great variations in sensitivity according to the stage of spermatogenesis irradiated (FRIESEN 1937, LÜNING 1952, AUERBACH 1954, ALEXANDER and STONE 1955, KHISHIN 1955, MOSSIGE 1955). Since genetic effects were sought and found quite early in these investigations, many of the experiments have had this aspect as an equally dominant motive.

Radioactive isotopes have been used extensively as mutagenic agents in microorganisms (HERSHEY, KAMEN, KENNEDY and GEST 1950, HUNGATE and MANNELL 1952, FUERST and STENT 1956).

The mutagenic or lethal effect may follow from the ionizations caused by the emitted radiation or by recoil of the daughter nucleus, or the chemical effect caused by valence change of the nucleus. According to the views of STRAUSS (1958) it is doubtful if the two last-mentioned effects may be separated, though their combined efficiency has been measured repeatedly (STENT and FUERST 1955, STRAUSS, VAHARU, FRICKEY and MATHESON 1956, CASTAGNOLI and GRAZIOSI 1957).

Investigators of radioisotope metabolism in insects have had many different aims. Thus, we find pure physiological distribution studies with tracer doses (e.g. BOWEN 1951), tagging experiments for ecological purposes (e.g. HOFFMAN and LINDQUIST 1951), studies of developing insects under chronic irradiation (e.g. BLUMEL 1950, ARNASON, IRWIN and SPINKS 1947) — and — finally — works on the genetic effects of radiation (BATEMAN and SINCLAIR 1950, ARNASON *et al.* 1951, KING 1953, and later). In several of these cases observations have been made on smaller scale in several of the above fields.

R. C. KING has studied many of the aspects of P^{32} metabolism and radiation effect in *Drosophila*. He has calculated the genetically significant dose in P^{32}-containing ♂♂ (1952), studied the retention of P^{32}

and estimated the genetically significant dose experimentally (1953), measured the RBE of P^{32} β-particles (1954 a), the turnover of phosphorus (1954 b) and the somatic radiation effect on developing *Drosophila* (1954 c), and — with L. P. WILSON — he has studied the radiation effect and the phosphorus balance in homogeneously labeled flies (KING and WILSON 1954, 1955). Numerous references will be made to his papers in the following, and some of the results will be subjected to further discussion.

BATEMAN (BATEMAN *et al.* 1950, BATEMAN 1955) has concentrated his efforts on the genetic aspects of the problem. His results after raising *Drosophila* larvae and pupae in media containing P^{32} are interesting enough, but the author is himself not satisfied that the questions are answered anywhere near fully, and his conclusion is that they never will be, since the material presents "almost insuperable difficulties". To quote, "It appears, then, that one can have no confidence in attributing any difference which has been or may in future be revealed between the two mutagens [i.e. X-rays and P^{32}] to the quality of the radiations (such as the transmutation of P^{32} to S^{32}). There are too many other possible sources of difference" (BATEMAN 1955, p. 197).

It is with these somewhat pessimistic views in mind the present report should be read. It is concerned with a series of experiments where both aspects of radioisotope characteristics have been utilized in physiologic and radiogenetic experiments with *Drosophila melanogaster*. In cognizance of the large number of unknown variables, it has been thought better to map these extensively, in order to provide a basis for fruitful working hypotheses, after which the more important variables may be studied in an intensive way in the future, rather than to embark upon a strictly quantitative study of an ill defined, narrow sector with unmapped interactions with the surrounding biological landscape. This principle also to some extent explains why often so many things have been done within one single experiment, sometimes necessitating the sacrifice both of parallels within the experiment and of replication.

The chronological sequence of experiments is not the same as that used in the presentation below. The first experiments were concerned solely with incorporation studies in different organs. A few genetic tests did, however, show that such effects were present and were measurable, and later experiments were largely planned and executed with both genetic and physiologic aspects in mind. The experiments with Y^{91} were planned only as genetic tests, and studies of the distribution of Y^{91} have followed. The theoretical evaluation of the combined P^{32} and Y^{91} radia-

tion effect data led to the need for more exact retention data for P^{32}, and to some of the experiments which are presented first in the following. In the presentation, the original Roman numerals identifying the different experiments have been used throughout.

The present series of experiments therefore have had three main goals. Firstly, the determination of the period needed for spermiogenesis — from the last premeiotic deoxyribonucleic acid (DNA) synthesis to the maturity of sperm from the same cells. Secondly, an evaluation of the radiation effect to be expected from the radioactive phosphorus ingested by the fly. And thirdly, an estimate of the mutagenic efficiency of the disintegration of P^{32} contained in the genetic material of *Drosophila* males. — In supplementary investigations the turnover of P^{32} in *Drosophila* ♂♂ has been studied, and these results will be presented first in the following. A detailed report on the distribution of radioactive yttrium after injection and ingestion of Y^{91} citrate is in preparation.

II. P^{32} METABOLISM IN DROSOPHILA ♂♂

In order to evaluate the results of the genetic tests and of the studies on incorporation of P^{32} into sperm, to be described below, it has been found necessary to study in some detail the retention and distribution of radioactive phosphorus after the administration of a single meal of P^{32}.

A. Materials and methods

1. Biological material

In all the present experiments ♂♂ from Canton S+wild type stock have been used. This stock has been maintained in the laboratory by mass transfer, but has been cleaned of spontaneous mutations by occasional brother-sister pair matings. All experimental matings have been with Muller-5 stock [1] virgin ♀♀, usually 2—3 days old. The stocks have been kept in a thermostat at $25° \pm 1$, on a standard *Drosophila* medium of cornmeal — molasess — dead brewer's yeast — agar, seeded with live yeast before use.

In the present experiments it has in all cases been the practice to give the males a single meal of P^{32}-containing solution. This technique was

[1] The well known Muller-5 stock has been used for all the recessive lethal tests in the present experiments. It contains an X-chromosome constructed by H. J. MULLER (SPENCER and STERN 1948). This chromosome has inversions preventing crossing-over, and the markers "white-apricot" and "Bar".

developed independently of, but is similar to the one recommended by HOFFMANN and LINDQUIST (1951) for tagging blowflies, and to GROSCH and SULLIVAN's (1953) method for feeding *Habrobracon*. It is different from that used by KING (1953), by BATEMAN and SINCLAIR (1950), and by ARNASON, IRWING and SPINKS (1947) in P^{32} work with *Drosophila*, and SUOMALAINEN, TURPEINEN and NIINI (1956) in their study of C^{14} radiation effects on *Drosophila*. In the latter case, the technique of feeding larvae on a radioactive medium for several days has certain advantages, since, because of the short β-particle range it is possible to calculate fairly exactly how much radiation has been absorbed by the gonads. When high energy beta-particles are involved, as in P^{32} work, leaving the larvae and pupae exposed has distinct disadvantages when dose calculations are to be attempted, since the geometry of the culture vessel, the site of pupation etc. will be important variables.

In the present experiments the following method has been used: The P^{32} solution is neutralized, with phenol red as indicator. About 0.3 grams of sugar are added per ml, and finally a small drop of India ink. The solution is mixed thoroughly, and a drop or two is then put on to a filter paper strip ($1 \times 1/2$ cm) which has been fixed to a small wooden spoon by means of paraffin. The final concentration of P^{32} in the feeding solution has been usually between 1.0 mC and 0.1 mC per ml. — Young flies are picked from the stock bottles and left in a dry shell vial until they recover from etherization. The spoon is put into the shell vial and the flies are left to feed. Aged flies are stored on normal yeasted medium and then shaken into an empty vial where the spoon containing P^{32} is introduced. After the feeding period it is easy to determine which of the flies have eaten, from the blackening of the crop seen through the ventral abdominal wall. The feeding period is usually about one hour, but down to a few minutes has been used in some experiments. — Washing the flies in dilute alcohol or in saline shows that the surface contamination of the fly is negligible after this type of feeding.

In the first experiments, the males were picked out by inspection after feeding, mated, and left for 24 hours, after which their radioactivities were measured, ranged and the final experimental distribution determined. Later, the activity measurements and the distribution of males to different groups have been made on Day 0, immediately after feeding. Thus, it is necessary to mate only those males needed for the experiment, and it is simpler to select a group of males with the most suitable range of activities.

For the measurements of radioactivity content, the males are put individually into No. 5 gelatin capsules, which are placed on Perspex holders which fit into aluminum sample holders provided with the scaler.

The males have been etherized as lightly and as rarely as possible, and apparatus has been designed for putting them into and taking them out of the capsules without any treatment (OFTEDAL 1956 a). When groups of males, or males with females, are to be handled, etherization has, however, been found unavoidable. The flies have usually been kept in shell vials with cotton stoppers. The food has been in most experiments the usual yeasted *Drosophila* medium. The phosphorus content of this medium is about 0.3 mg per gram (KING 1953), but the flies mainly eat yeast, the phosphorus content of which is 5.55 mg per gram (KING and WILSON 1954).

In some experiments the flies have been kept on a minimal medium, consisting of honey diluted about 1 to 50 with water. The food has been offered either from cellulose soaked in the solution, or from special small feeder vials inserted into the shell vial. The phosphorus content of undiluted honey is about 160 μg per gram (SHERMAN and LANFORD 1957). Evaporation from the cellulose surface may cause local concentration of the solution. — A *Drosophila* ♂ assimilates normally about 0.7 μg phosphorus per day (KING 1954 b). Since a big meal is about 0.05 mm^3, and not many more than ten such meals may be expected in a day, phosphorus intake on honey is certainly not higher than 0.08 μg per day. It therefore seems safe to assume that ♂♂ on honey are on a phosphorus deficient diet. The flies can live for a long time under these conditions, but the ♂♂ tend to go sterile earlier than do ♂♂ on a normal yeast diet.

In some experiments the males have been kept without females, in others they have been mated continuously. In the latter case, it has been sought to provide an excess of females, so that no accumulation of sperm should take place at any time (FRIESEN 1937, MOSSIGE 1955). This means that males were provided with five 2—3 day old virgin females each every day for the first six days. After this time, three females were used for each change. After 11 or 12 days, females were changed only every other or every third day.

When measuring the radioactivity transferred from males to females by mating, all the females mated to one male for one period have been put into one gelatin capsule for measurement. In productivity measurements, all the females from one mating period with one male are put into one half-pint bottle. After five days, the females are removed from

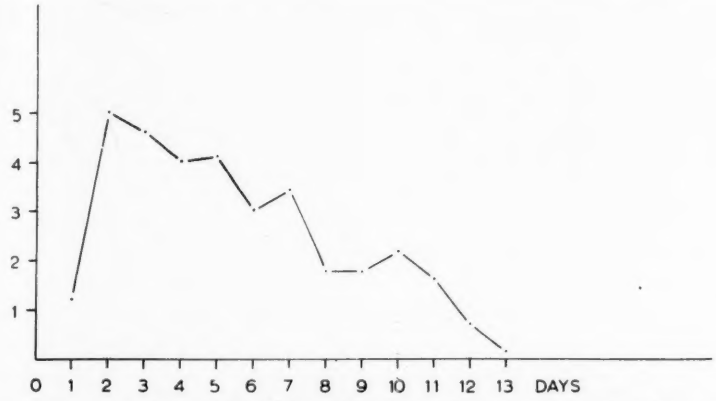

Fig. 1. Average number of M-5 ♀♀ inseminated per ♂ per day, determined by microscopic examination of seminal receptacles.

the bottle and extra yeast added as cellulose soaked in a milky suspension of live yeast. The number of offspring has been scored usually 12 and 16 days after mating. Very few flies eclode after the twelfth day.

The mating capacity of the males has been checked by productivity measurements of individual females, and by dissection and microscopic inspection of the seminal receptacles of females at the end of the mating period. Findings in one dissection experiment are shown in Fig. 1. It will be noted that the number of ♀♀ containing sperm from one ♂ falls from 5 on Day 2 to about 3 on Days 6—7. On Day 8 the number is below two and this level is maintained till Day 11, after which the number again falls rapidly to near zero on Day 13. The fertility after this time has not been investigated in the present experiments, but it is known (MOSSIGE, personal communication) that some ♂♂ regain a fairly high level of fertility, while others go completely sterile.

In the genetic experiments, heterozygous F_1 ♀♀ have been tested for complete absence of wild type male offspring. In the graphical presentation of the genetic results, the 95 per cent expectation intervals are presented as calculated with STEVENS' correction (FISHER and YATES 1953).

2. Calibration of counter equipment

In earlier measurements of radioactivity different counter assemblies were used, but after Exp. XL an assembly consisting of an end window Geiger-Müller counting tube with Frieseke & Hoepffner scaler, time

printer and sample changer has been used. Different tubes have been used, from different manufacturers. The efficiency of the counter assembly has been normalized by measuring a standard P^{32} simulating source in connection with the sample measurements. The source has been filtered down to a measurable activity of about 5000 cpm, and this figure has been selected as an arbitrary standard of efficiency. This practice was instituted only after a certain experience had been gained. Generally, the efficiency has been somewhat higher, and the sample values have been reduced accordingly.

The scaler assembly has been calibrated in absolute values with the kind help of Professor ALEXIS PAPPAS, of the Nuclear Chemistry Division, University of Oslo. A number of aliquots of a weak P^{32} solution were pipetted on to aluminium sheet of about 2 mg/cm^2 weight. These samples were mounted with minimal back scatter, and measured in one of Professor PAPPAS' counters where the geometry factor had been calculated theoretically, and checked by measurements of Sr^{90}—Y^{90} standards. From the results, the absolute numbers of disintegrations in the P^{32} aliquots have been calculated, after corrections for difference in energy between P^{32} and Sr^{90}—Y^{90}, and for differences in geometry (PAPPAS 1953). After these measurements, the samples have been remounted on lead plaquettes and measured in the scaler assembly used in the present investigations. The increase of measurable radiation on account of back scatter should in this case be of the order of 80 per cent. The geometry factor for the scaler assembly standardized to 5000 cpm from the P sim source has been determined by comparison with the absolute values as calculated from Professor PAPPAS' measurements. An average efficiency of 34.76 ± 0.45 per cent obtains for samples mounted on lead. With a different G-M tube used during a subsequent period an efficiency of 37.91 ± 0.75 is found.

The efficiency of the scaler assembly for measuring radioactivity contained in live flies is considerably lower than when the radioactive material is mounted on lead. This difference has been determined in several different ways. In the first method, flies with known radioactivity content are homogenized in a drop of water, and the suspension is evaporated to dryness on a lead plaquette. Measurements then give the difference in efficiency directly, after corrections for background, dead time and decay losses. Self absorbtion may be ignored. The results from one such experiment involving 8 flies show an average efficiency of 239.8 ± 9.4 per cent of lead mounted over live fly measurements.

Secondly, flies with known radioactivity content have been killed, and dissected without use of immersing fluid into parts consisting of wings, legs, head, thorax and abdomen. These are then mounted on lead plaquettes by means of a thin solution of Euparal. The sum of the corrected activities on the plaquettes is then compared with the earlier measurements of live flies. In two experiments involving 16 flies, an average efficiency of 242.2 ± 2.12 per cent of lead mounted over live fly measurements obtains. Since the preparations are dehydrated with absolute alcohol during mounting on the plaquettes, self absorption is reduced and has been ignored. A value of 240 per cent has been used in the subsequent calculations. — The counter was kept running continuously during experiments, measurements of background being run when fly measurements were not in progress.

3. Variation and error in radioactivity measurements

In Figs. 2 and 3 are shown the results of a short time experiment involving one group of 14 ♂♂ aged two days at the time of feeding, and followed non-mated for 36 hours. In Fig. 2 are recorded the measurements of the individual ♂♂, while Fig. 3 shows the smoothed curves used in the calculations, and also that for a similar group of ♂♂ less than 3 hours old at feeding.

In Fig. 2 there appears to be a great spreading in the measurements as early as 3 hours after feeding, but this range is essentially maintained for the rest of the series. This is partly a false presentation, since inexactitude in the measurements is most pronounced at the first measurement, when a large number of males with highly different activities is to be measured in as short a time as possible. In subsequent measurements, the number of ♂♂ is limited to the experimental group, and the activities of the individual males are known within limits, so that the counting times may be adjusted accordingly. This may be demonstrated by calculating the standard error of the group when the measurements are expressed as percentages of 0 hour measurements, or of e.g. 3 hour measurements. Taking the 9 hour measurement of young ♂♂ as an example, we find a mean retention with standard error of 77.8 ± 2.55 when referring to 0 hours as 100 per cent, and correspondingly 88.0 ± 1.42 when referring to 3 hours as 100 per cent. The error has thus been reduced from 3.28 to 1.61 per cent of the mean.

A true source of error and variation — in addition to the statistical ones — stems from a combination of geometrical and biological factors. After the flies' recovery from etherization, they move about in the

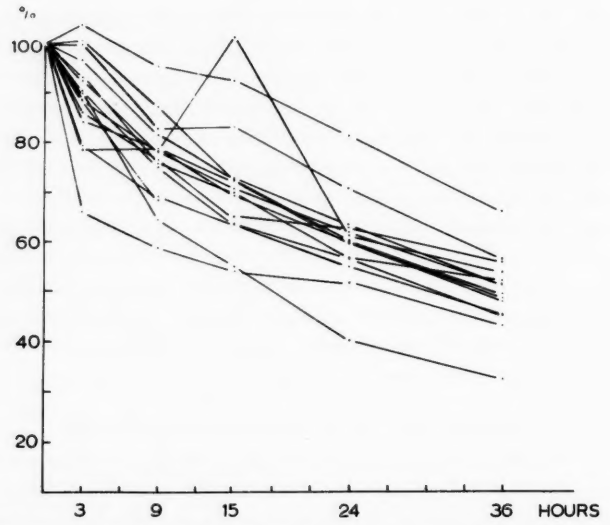

Fig. 2. P^{32} retention in ♂♂ during the first 36 hours after a meal (CVII). Individual measurements of 14 ♂♂. Initial P^{32} content 0.032—0.011 μC per ♂.

capsule, and although small — the distances involved will influence the counting rate. Also, sometimes the flies will remain in the capsule for as long as a couple of hours, and radioactive excreta will then contaminate the capsule. Although the total amount contained in the fly+ capsule will not change, the positioning of the capsule will be important. — Since the capsules become contaminated in this way they are discarded after use.

Fig. 2 illustrates one further point. It will be seen that at 15 hours there is one measurement which is probably too high (104 per cent), and this is attributed to some unidentified fault in the electronic circuits. In about 250 measurements in 36 hours, this was the only obviously incorrect measurement. In some periods, the standard has been better than this. At other times, the scaler has been less reliable even than the flies.

In Fig. 3 a final point deserves mention. It will be seen that there appears to be a slight difference in the retention in the two groups at 24 and 36 hours, old males retaining some 3 per cent more of the original activity than young males do. Although this difference in all probability is not significant, biologically as well as statistically, it serves

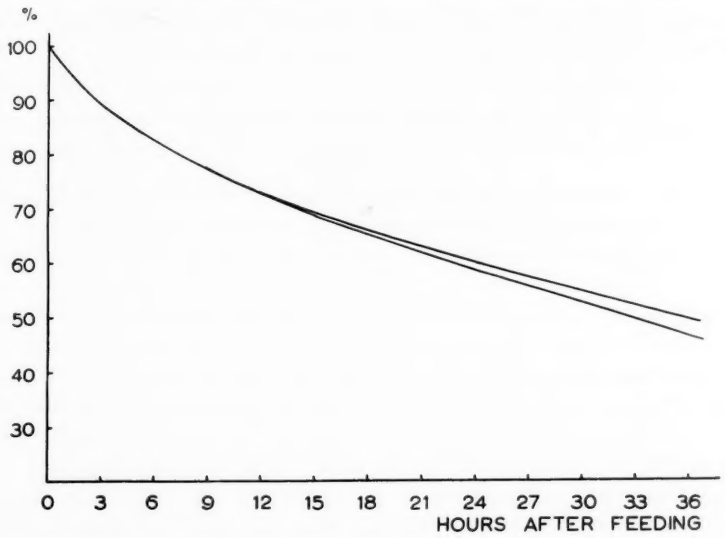

Fig. 3. Retention of P^{32} in flies during the first 36 hours after feeding (CVII).

Upper curve: Average retention for 14 ♂♂ 2 days old at feeding. Non-mated before feeding. Initial P^{32} content 0.032—0.011 μC. Lower curve: Average retention for 15 ♂♂ <3 hours old at feeding. Initial P^{32} content 0.053—0.022 μC.

Not corrected for radioactive decay.

to illustrate a difficulty encountered in evaluating data of this type. Since the measurements at different times involve the same group of males, the measurements cannot be taken as independent estimates of the decay of radioactivity in a large population of flies from which samples are drawn at the different times. The statistical methods commonly used to evaluate differences between curves of this type are based on an assumption of independent samples drawn from a large population at different times, and they may therefore not be applied to the present material without considerable modification. A more refined statistical analysis of the significance of differences between groups has therefore not been made. In some cases such refined treatment appears superfluous, since the differences are large and consistent. In other instances the information to be gained has not been thought valuable enough to justify the extra effort involved. The converse solution, that of increasing group size sufficiently to make independent measurements

possible, would, on the other hand, introduce experimental difficulties which would more than obviate the gains to be expected.

B. Experiments on P^{32} retention

1. Introduction

The P^{32} content in flies after feeding males a single meal of P^{32}-adulterated sugar solution has been followed over different periods for different purposes. The following types of experiments are discussed below:

a) Feeding $<$ 3 hour old males, and following them for up to 30 days on normal medium or on honey, mated or non-mated, in order to follow P^{32} turnover under different conditions. (E.g. Fig. 4.)

b) Feeding $<$ 3 hour old males, and following them for 10—15 days with standard mating, in order to determine the cumulative dose to the testes during the whole period. (E.g. Fig. 32.)

c) Feeding 2—3 day old males, and following them for 24—36 hours, for the purpose of determining the relative dose received by the mature sperm in the testes, in order to measure the fraction of the released radiation energy which is absorbed in the testes. The males were not mated. (E.g. Fig. 3.)

d) Measuring activity in the females at the end of each mating period, in order to determine P^{32} transfer from radioactive males under different experimental conditions. (E.g. Fig. 15.)

e) Measuring activity in females at the end of each mating period, after hydrolysis in HCl, in order to determine incorporation of P^{32} into sperm. (E.g. Fig. 24.)

In a) and d) all measurements have been corrected for radioactive decay after the time of feeding. In b) and c) no correction for radioactive decay is used, since it is the amount of radiation emitted which is of interest. In e), the measurements have been corrected for decay after Day 0, but with some doubt. Strictly, the correction should involve only the time between the moment of incorporation of the radioactive atoms into the sperm, and that of measurement. Since this time is not known exactly, it has been thought more correct to follow the usual practice.

Two points should perhaps be mentioned in connection with the retention experiments. Firstly, the P^{32} in different experiments has been obtained either from Institutt for Atomenergi, Kjeller, Norway, or from The Radiochemical Center, Amersham, U.K., as phosphate in acid solution, and usually with phosphorus carrier already added. The amount

TABLE 1. *Initial range of isotope content per ♂ in different groups in various retention experiments in μC.*

Experiment	Non-mated ♂♂ on honey	Mated ♂♂ on honey	Non-mated ♂♂ on yeast	Mated ♂♂ on yeast
LXVI	0.193—0.039	0.200—0.033		
LXXIII	0.085—0.011		0.083—0.014	
LXXV	0.148—0.091	0.139—0.066	0.139—0.067	0.139—0.080
XCVI	0.080—0.023	0.090—0.022	0.101—0.023	0.069—0.028

of carrier has not been measured, and is bound to have differed between batches. This may have influenced certain of the results obtained to some extent.

Secondly, the amount of P^{32}+carrier ingested by the ♂♂ in different experiments have not been constant, since for different purposes different levels of activity were desirable. However, within a 20—30 fold range in initial activity, no systematic differences in retention pattern have been discerned. The initial amounts of P^{32} ingested by ♂♂ are given in Table 1 for the life retention experiments, otherwise in the text to the illustrations.

It should perhaps again be emphasized that the primary purpose of the studies to be reported are to be found in relation to the radiation effects. It is for this reason that in some aspects the retention and turnover studies are inadequate, e.g. as regards the evaluation of total amounts of phosphorus involved. The results discussed below are therefore largely limited to problems which can be approached in a relative way — by intergroup comparisons, and to those solely concerned with radioactive phosphorus.

2. Life retention

In measuring the retention of P^{32} over longer periods, it has been usual to measure radioactivity content daily for the first 3 or 4 days, and every third to fifth day thereafter. The duration of the experiments differs, depending to some extent upon the success in keeping the flies alive. Retention has been measured in non-mated ♂♂, and in ♂♂ mated according to the system used in the genetic experiments (5 fresh ♀♀ per ♂ per day). In order to ascertain to what extent retention was influenced by diet, parallel experiments have been made with similar

groups of flies maintained on a diet of dilute honey, and on ordinary yeasted medium.

The statistical treatment of the observations has been limited by the small number of flies in each group. These numbers have been determined by the time needed for measurements, and for handling and mating the flies. However, the reproducibility of trends and differences between groups has been found good, even where absolute figures have differed between experiments.

In one experiment (XCVI), retention has been followed for up to 30 days. The curves have — after correction for radioactive decay — been analyzed further. The analysis follows the general pattern used by KING (1954 b). Since retention has been measured over longer periods, an extension of the data has been possible, but differences in feeding method do not make the results strictly comparable. Experiment XCVI will be used as a basis for discussion, with other data being introduced as needs arise.

About 150 Canton-S wild type ♂♂ < 3 hours old were allowed to feed for about 1 hour on a sugared P^{32} solution. After the feeding period, all ♂♂ with a well filled crop were put in gelatin capsules and their radioactivity measured. Of the 69 ♂♂ that were thus picked out, 60 were used and ranged into six groups of 10 ♂♂ each. The activities varied between 32624 cpm and 7014 cpm (0.101 μC—0.022 μC). Of the six groups, two were kept mated and two non-mated on a honey diet, while one mated and one non-mated group were kept on normal yeasted medium. All live flies were measured on Day 0, 1, 2, 3, 6, 9, 13, 17, 23, 27, 30. Measurements could not be performed on Day 20 on account of repairs of a radium gun being performed in a neighboring building.

The results from XCVI and three other experiments have been summarized in Table 2.

In the mated groups, the ♀♀ were measured for radioactivity at the end of the mating period, and were then put into culture bottles for offspring production as described above. Survival of ♂♂ varied greatly between groups, mainly because fermentation in the honey solution caused overflow and drowned some ♂♂. In Table 3 are given numbers of flies alive, and also numbers of fertile matings in the two mated groups.

From the table it will be seen that the ♂♂ on yeast are fertile longer than those on honey, and this observation has been corroborated in other experiments. Also, there appears to be a slightly better survival in the non-mated than in the mated groups (the great reduction in honey

TABLE 2. *Average amount of P^{32} in groups of non-mated (n-m) and mated (m) ♂♂ maintained on a diet of honey or yeast, expressed as per cent of each ♂'s initial P^{32} content, in four different experiments.*

In columns headed "♀♀" are given average amounts of P^{32} in ♀♀ mated to the corresponding group of ♂♂, expressed as *pro mille* of each ♂'s initial P^{32} content. After Day 11 two-day mating periods have been used. — The day 7 measurements of LXXIII are obviously erratic.

Days	LXVI			LXXIII		LXXV						XCVI					
	Honey			Honey	Yeast	Honey			Yeast			Honey			Yeast		
	n-m	m	♀♀	n-m	n-m	n-m	m	♀♀	n-m	m	♀♀	n-m	m	♀♀	n-m	m	♀♀
1	68.1	63.7	26.9	77.9	64.6	83.7	83.2	20.2	68.4	63.5	14.1	83.1	77.9	18.6	62.1	63.5	6.47
2			13.7	71.1	49.1	81.0	80.3	13.1	59.8	52.6	7.27	77.3	67.9	14.9	41.7	40.7	4.35
3	58.8	51.9	6.52			78.7	73.5	4.9	52.6	42.1	5.78	74.5	67.0	4.54	35.4	31.4	3.45
4			6.22	58.3	31.9	77.5	68.7	13.8	47.3	34.9	4.86			5.83			2.15
5			7.38											6.08			0.98
6	55.8	41.1	4.41									65.1	46.6	6.84	23.7	17.5	1.77
7			4.45	62.0	30.9	74.6	58.7		37.4	22.6				3.78			0.42
8			3.73			75.9	57.9		33.6	19.0				2.77			0.10
9	52.9	36.0	3.56			73.9	53.8		31.3	16.6		60.5	41.6	3.68	17.6	10.9	1.14
10			2.21											2.37			0.06
11			1.96			72.1			26.7	13.8							0.12
12	53.4	34.1															
13			2.25										34.0	1.11	11.5	6.7	0
14				52.7	15.3	68.5			22.7	9.9							
15														1.03			0
17													29.4		9.4	5.0	
23													26.1		7.3	2.9	
27													24.4		5.6		
30													28.4		6.1		

TABLE 3. *Fertile matings/males alive in Exp. XCVI.*

Mating period	1	2	3	4	5	6	7	8	9	10	11	12-13	14-15	16-17	18-20	21-23	24-27	28-30	alive after 30 days
H. m.	19/20	19/20	18/20	16/19	16/18	15/17	12/16	11/12	9/10	2/8	2/7	3/6	3/6	0/5	0/5	0/4	0/3	0/2	2
H.n.-m.	20	20	20			19			16		3	3		3		3	3	2	2
Y. m.	9/10	8/8	7/8	7/8	6/8	4/7	3/6	6/6	5/6	6/6	6/6	5/6	3/6	3/6	2/2	2/2	2/2	2/2	0
Y.n.-m.	10	10	9			9					9	8		7		6	5	5	5

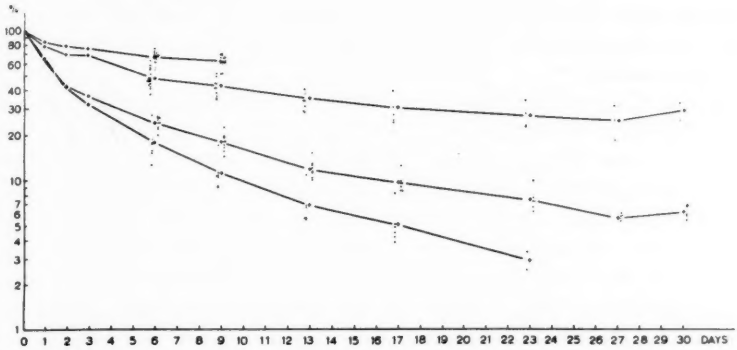

Fig. 4. Retention of P^{32} in non-mated and mated ♂♂, kept on honey and on yeast. (XCVI.) Semilog scale.

Upper curve: Non-mated on honey. Upper middle curve: Mated on honey. Lower middle curve: Non-mated on yeast. Lower curve: Mated on yeast. Dots indicate individual observations, crosses group averages.

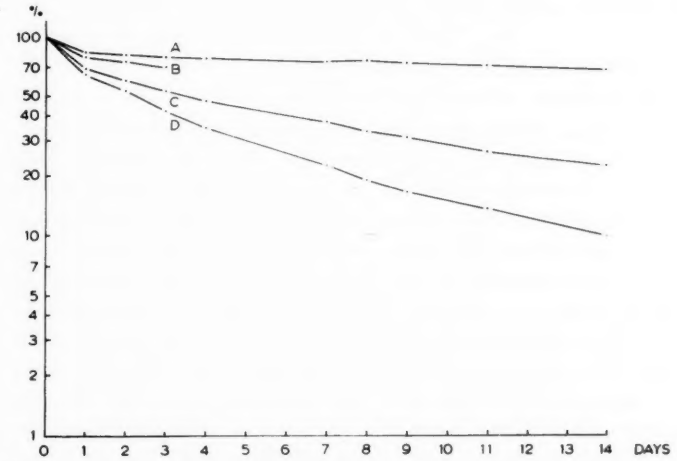

Fig. 5. Average retention of P^{32} after ingestion in non-mated and mated flies, kept on honey and on yeast. (LXXV.) Semilog scale.

Curve A: Non-mated on honey. Curve B: Mated on honey. Curve C: Non-mated on yeast. Curve D: Mated on yeast.

non-mated on Day 11 is because of drowning), but since the non-mated flies were handled less than the mated ones, the difference is probably not meaningful.

In Fig. 4 are shown the average retention curves for the four groups of ♂♂ in Exp. XCVI. It will be seen that there are characteristic differences between the groups, and that they are in the direction which would be expected in view of the experimental conditions. The group having the lowest retention is the mated group on yeasted medium. Next lowest is the non-mated group on yeast, showing that the diet is more important than the mating in determining phosphorus retention. The next highest retention is shown by the mated flies on honey, while the non-mated flies on honey have a very low and nearly constant rate of excretion per day after the first couple of days. This latter curve has not been carried further than Day 9 since only 3 flies survived after that day.

Similar curves from one other experiment (LXXV) are shown in Fig. 5. It will be noted that the same general pattern obtains, although there are differences in magnitude at all points. The reasons for these differences may probably be found in differences in specific activity in the P^{32} solution used, as well as in details of experimental conditions. The data are not extensive enough for hard and fast conclusions, and the deductions to follow are quantitatively very tentative.

3. Discussion

a. *Group analyses*

The retention curves have been analyzed in the following way:

From Figs. 4 and 5 it will be seen that when the average retentions for each of the four groups are plotted on semilog paper, the curves beyond a certain point are approximately straight lines. For each group, the line of best fit for this part of the curve has been calculated by the method of least squares on the basis of all individual observations on the relevant days (L_1). This line has been extended to Day 0, and the calculated values for the remaining part of the curve have been subtracted from the observed averages. On semilog paper, the resultant curve will again consist of a straight part and a curved part, the latter usually extending only over Day 0 and Day 1. Calculation of the second straight line, extension to Day 0, and subtraction then leads to new values for two or three points, to which a third straight line is fitted. Each of the three straight lines will represent an exponential function, and the sum of these functions will describe the P^{32} retention of the group.

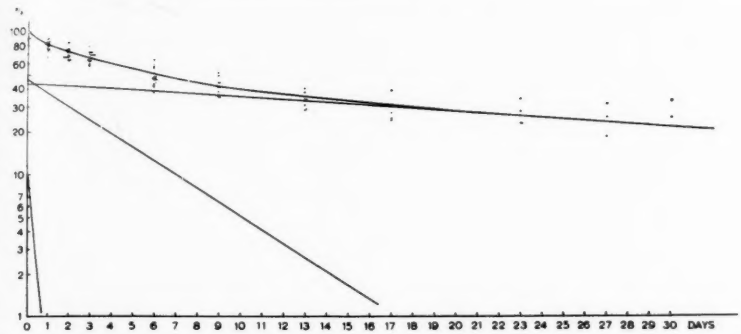

Fig. 6. Retention of ingested P^{32} in mated ♂♂ kept on honey (XCVI). Semilog scale. Dots represent observations. The curved line is the resultant of the three straight lines.

The resultant curve constructed from the straight lines will be described by an equation of the form

$$f_{(ret)} = i_1 e^{-k_1 t} + i_2 e^{-k_2 t} + i_3 e^{-k_3 t}$$

In Figs. 6, 7 and 8 are given examples of this procedure, and it will be seen that the resultant composite curve gives a satisfactory fit to the observed points. In Table 4 are given the equations for the different groups in several experiments.

In Fig. 9 upper part is shown the relative magnitude of the intercepts in the different groups of Exp. XCVI. It will be seen that the slow pro-

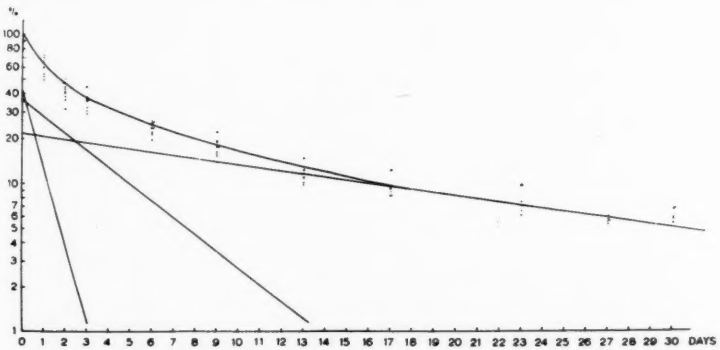

Fig. 7. Retention of ingested P^{32} in non-mated ♂♂ kept on yeast (XCVI). Semilog scale.
Dots represent observations. The curved line is the resultant of the straight lines.

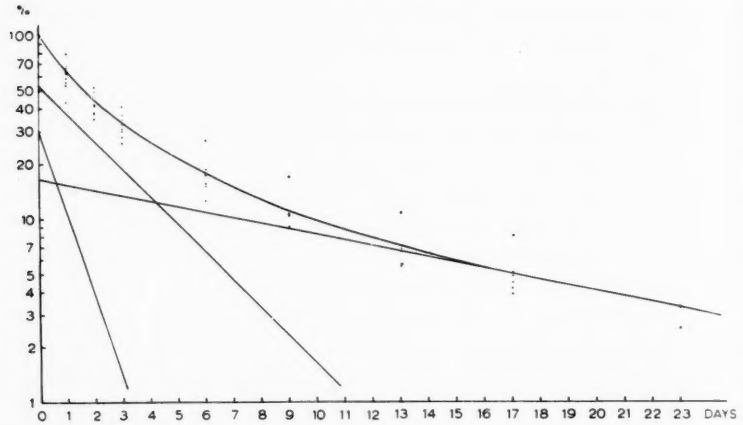

Fig. 8. Retention of ingested P^{32} in mated ♂♂ kept on yeast (XCVI). Semilog scale. Dots represent observations. The curved line is the resultant of the straight lines.

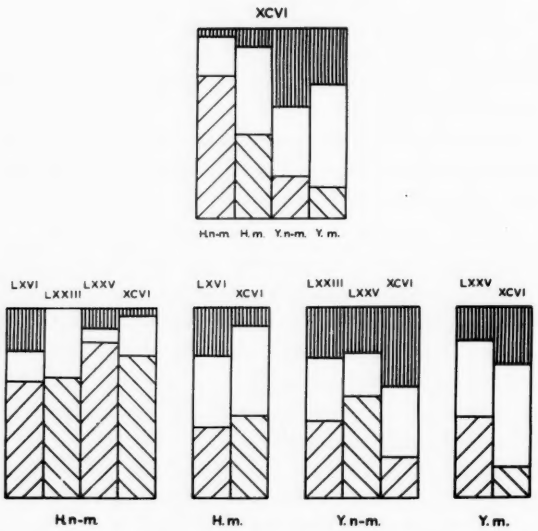

Fig. 9. Y-intercepts of the different processes in groups non-mated and mated, on honey and on yeast.
Oblique lines: Slow process. No lines: Medium process. Vertical lines: Fast process. The sum of the three intercepts is 100 %.

TABLE 4. *Retention equations.*

XCVI.

Non-mated ♂♂ on honey. Basis for L_1 Days 6 and 9. 16 flies, 32 measurements.
$Y=74.69\cdot e^{-0.0234t}+21.00\cdot e^{-0.544t}+4.31\cdot e^{-\sim 10t}$

Mated ♂♂ on honey. Basis for L_1 Days 17, 23, 27. 4 flies, 11 measurements.
$Y=43.44\cdot e^{-0.0233t}+46.69\cdot e^{-0.228t}+9.87\cdot e^{-\sim 6.9t}$

Non-mated ♂♂ on yeast. Basis for L_1 Days 13, 17, 23, 27. 7 flies, 25 measurements.
$Y=21.65\cdot e^{-0.0477t}+36.55\cdot e^{-0.261t}+41.78\cdot e^{-1.14t}$

Mated ♂♂ on yeast. Basis for L_1 Days 13, 17, 23. 6 flies, 14 measurements.
$Y=16.24\cdot e^{-0.0698t}+53.46\cdot e^{-0.351t}+30.10\cdot e^{-1.03t}$

LXXV.

Non-mated ♂♂ on honey. Basis for L_1 Days 4, 7, 8, 9, 11, 14. 9 flies, 54 measurements.
$Y=81.83\cdot e^{-0.0121t}+7.19\cdot e^{-0.924t}+10.98\cdot e^{-\sim 10t}$

Mated ♂♂ on honey. Not analyzable.

Non-mated ♂♂ on yeast. Basis for L_1 Days 9, 11, 14. 9 flies, 27 measurements.
$Y=53.34\cdot e^{-0.0624t}+22.66\cdot e^{-0.306t}+24.11\cdot e^{-2.71t}$

Mated ♂♂ on yeast. Basis for L_1 Days 9, 11, 14. 4 flies, 12 measurements.
$Y=42.58\cdot e^{-0.103t}+39.91\cdot e^{-0.408t}+17.51\cdot e^{-\sim 7.5t}$

LXXIII.

Non-mated ♂♂ on honey and on yeast.

Evaluation by eye, and graphical quantization:

Non-mated ♂♂ on honey.
$Y=63.5\cdot e^{-0.011t}+36.5\cdot e^{-0.73t}$

Non-mated ♂♂ on yeast.
$Y=40.5\cdot e^{-0.064t}+33.0\cdot e^{-0.44t}+26.5\cdot e^{-1.64t}$

LXVI.

Non-mated and mated ♂♂ on honey.

Non-mated ♂♂ on honey. Basis for L_1 Days 6, 9, 12. 9 flies, 27 measurements.
$Y=61.53\cdot e^{-0.00696t}+16.15\cdot e^{-0.475t}+22.32\cdot e^{-3.28t}$

Mated ♂♂ on honey. Basis for L_1 from non-mated group.
$Y=37.58\cdot e^{-0.00696t}+37.10\cdot e^{-0.253t}+25.32\cdot e^{-3.51t}$

cess is more important on honey than on yeast, and also more important in non-mated than in mated groups. The medium slow process is more important on yeast than on honey, and more important in mated

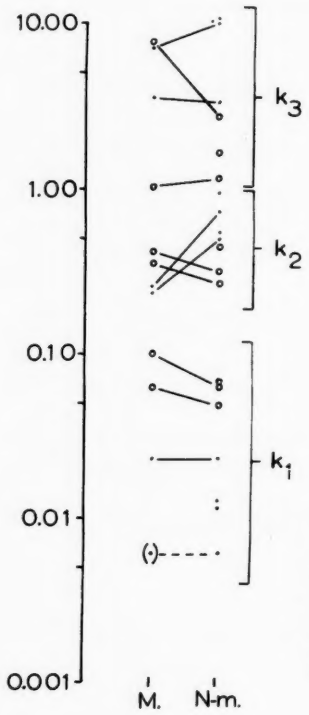

Fig. 10. Numerical values of the exponents of the equations describing life retention.
k_1=Slow process. k_2=Medium process. k_3=Fast process. M.=mated ♂♂; N-m.=non-mated.
Circles indicate groups kept on yeast, dots groups kept on honey. Lines connect corresponding groups in the same experiment. Log scale.

than in non-mated groups. This pattern has been repeated qualitatively in other experiments involving some or all groups, but the quantitative aspect is less satisfactory. Again, it is suspected that among the uncontrolled experimental conditions, the different amounts of carrier in the various P^{32} solutions may be the most important. In the lower part of Fig. 9 are shown the relative magnitudes of the corresponding intercepts in similar groups in several experiments. Although there is considerable variation, the tendency appears to be the same as that seen in Exp. XCVI.

In Fig. 10 are shown the numerical values of the exponents of Table 4 plotted on a log scale.

The exponent of the slowest component of the equation is obviously most strongly influenced by the diet, and in the direction that the turn-

over in the flies on honey is slower by a factor of maybe 3. Whether the ♂♂ have been mated or not is unimportant in the group on honey (one comparison only), but mated flies on yeast have an exponent which may be about 1.5 times that of the non-mated flies.

The exponent of the medium component of the equation is influenced by the diet to a certain extent, and in both groups also by mating. In flies kept on honey the value of this exponent will be higher if they are not mated, while in flies kept on yeast the value will be higher if the flies are mated. The last exponent — that of the fast component — has been determined on the basis of fewer observation points and presumably with a much greater error than the others, and shows correspondingly greater variation. However, it appears as if it is comparatively little influenced by mating, but strongly by diet, flies on honey having a higher value than those on yeast.

In sum, on yeast mating leads to a more rapid turnover of both the slow fraction by a factor of 1.5, and the medium fraction by a factor of 1.3, the fast fraction turnover possibly being uninfluenced. On honey, on the other hand, the slow fraction turnover is not influenced by mating (one comparison), while the medium fraction turnover in mated flies is reduced to half that in non-mated flies. The third fraction turnover is again uninfluenced by mating.

We therefore have the paradoxical situation that whereas mating leads to a more rapid rate of turnover of P^{32} in the flies kept on yeast, it lowers the turnover rate in flies kept on honey. The finding of a higher total rate of excretion in mated than in non-mated flies on honey is, of course, explained by the greater fraction of the total P^{32} excretion which goes over the medium and the fast type of process in mated flies in comparison with the non-mated ones.

It is tempting to interpret the different exponential functions as describing averages of different physiological processes, and a theoretical model of this kind may be useful in some respects. However, it is obvious that the present model is not sufficiently refined to provide a meaningful picture. Therefore, it is not possible to ascribe definite physiological processes to the different members of the retention equation, nor should the different Y intercepts be taken to represent phosphorus pools with little intercommunication. In most respects, it therefore will be more profitable to base the comparison between groups on the original experimental curve, instead of on the different exponential functions.

b. *Intergroup comparisons*

The influence on P^{32} retention of a honey versus a yeast diet may be examined by comparing similar groups on the two diets. Correspondingly the influence of mating may be shown by comparing non-mated and mated groups on the same diet. We therefore have four different comparisons which can be made.

Taking the influence of diet first, this can be shown by expressing for each day the retention in the yeast group as a fraction of the retention in the corresponding honey group. In Fig. 11 are presented curves

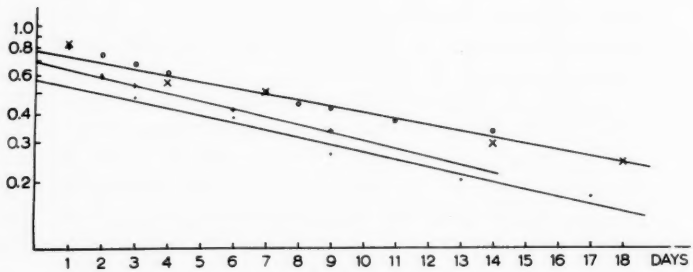

Fig. 11. Retention of P^{32} in yeast groups plotted as fraction of retention in honey groups. Semilog scale. Lines fitted by method of least squares.
Upper curve ×: non-mated ♂♂ (LXXIII), ○: non-mated ♂♂ (LXXV). Middle curve +: non-mated ♂♂ (XCVI). Lower curve ●: mated ♂♂ (XCVI).

derived from four such calculations. The upper two curves coincide and represent two independent experiments performed with non-mated flies on honey and on yeast, but with P^{32} from the same batch (LXXIII and LXXV). The lower curves are calculated from the retention in non-mated (higher values) and mated (lower values) flies on honey and on yeast, all four groups from the same experiment (XCVI). It will be noted that when plotted on semilog paper all four curves have a predominant linear section which commences as early as Day 2—3. The linear fit is not as good in the comparison between the mated groups as it is for the non-mated groups. The slopes of the lines are quite similar, and it seems probable that there is no real difference between them. The differences in Y intercepts are presumably indicative of real differences. The equations for the linear parts of the curves are given in Table 5.

TABLE 5. *Mathematical expression for the curves in Figs. 11 and 12.*

LXXIII and LXXV	Yeast non-mated / Honey non-mated	$Y = 0.773 \cdot e^{-0.0656\,t}$	Fig. 11
XCVI	Yeast non-mated / Honey non-mated	$Y = 0.684 \cdot e^{-0.0820\,t}$	
XCVI	Yeast mated / Honey mated	$Y = 0.568 \cdot -0.0755\,t$	
LXXV	Yeast mated / Yeast non-mated	$Y = 1.014 \cdot e^{-0.0709\,t}$	Fig. 12
XCVI	Yeast mated / Yeast non-mated	$Y = 1.091 \cdot e^{-0.0636\,t}$	

From these results it appears that the ratio of the retentions in flies kept on yeast and on honey decreases — after the first two or three days — exponentially with time in the same way in mated and in non-mated flies.

If on the other hand, similar comparisons are made between mated and non-mated groups kept on the same diet, by expressing the retention in the mated group as a fraction of that in the non-mated group, the results are not as clear cut. Five such comparisons have been made, two on yeast and three on honey. The yeast curves when plotted on semilog paper are straight lines and parallel each other over Days 1—9,

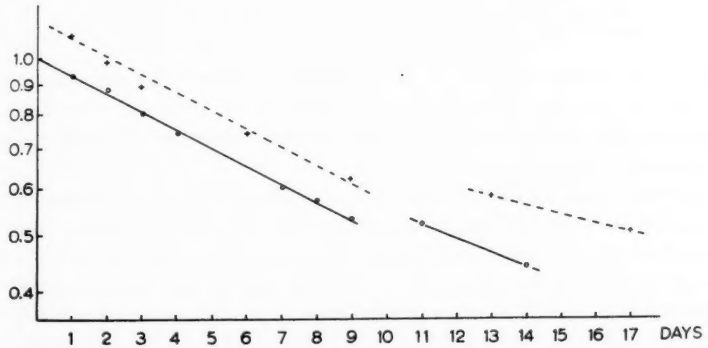

Fig. 12. Retention of P^{32} in mated ♂♂ expressed as fraction of retention in non-mated ♂♂, both on yeast. Semilog scale. Lines fitted by method of least squares. Upper curve: XCVI. Lower curve: LXXV.

but again with a difference in the Y intercepts (Table 5, Fig. 12). In both curves there is a break after Day 9, while the curves after Days 11—13 seem to continue with a slope somewhat similar to that found between Days 1 and 9. Thus, there is in the yeast groups an exponential decrease in the ratio of the retentions in the mated and in the non-mated groups with time, and this relationship is essentially constant up to Day 9. It is tempting to associate the break in the curves following Day 9 with the minimum in offspring production and mating frequency observed at this time in these as well as in other experiments.

The fourth comparison possible, that between mated and non-mated

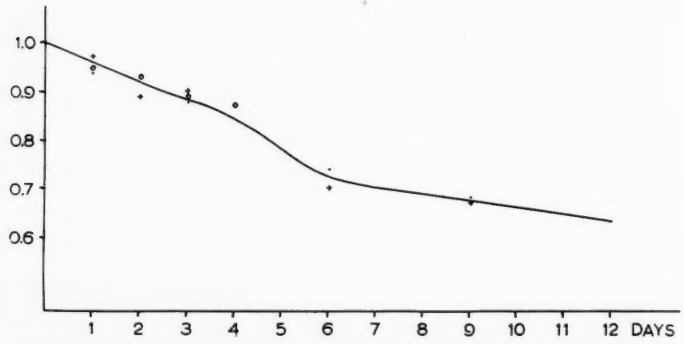

Fig. 13. Retention of P^{32} in mated ♂♂ expressed as fraction of retention in non-mated ♂♂, both on honey. Line fitted by eye.
●=LXVI. ○=LXXV. +=XCVI.

groups on honey, should — by necessity — show also an exponential change in the ratio between groups. This is not the case, and the relationship appears rather more complex. The values are therefore plottet on a linear scale in Fig. 13.

It is not clear if in the last comparison by chance the experimental deviations accentuate each other, and the relationship really is exponential in this case, or if the fitting of exponential functions to the other comparisons has not been permissible. It appears as if the comparison between mated ♂♂ on the two diets is the most suspect (Fig. 11, lower curve), and since the survival, mating transfer and productivity are more variable in the group on honey than in that on yeast, it may well be that it is this group which shows a less predictable retention pattern.

c. *Mating loss, mating transfer and productivity*

If we next consider the amounts of radioactivity which the ♂♂ lose in conjunction with the mating process, these amounts may be registered by measuring the excessive loss in the mated ♂♂. Part — if not all — of this loss may be traced also by measuring the amounts deposited in the mated ♀♀.

If the retention values for mated flies are subtracted from those of

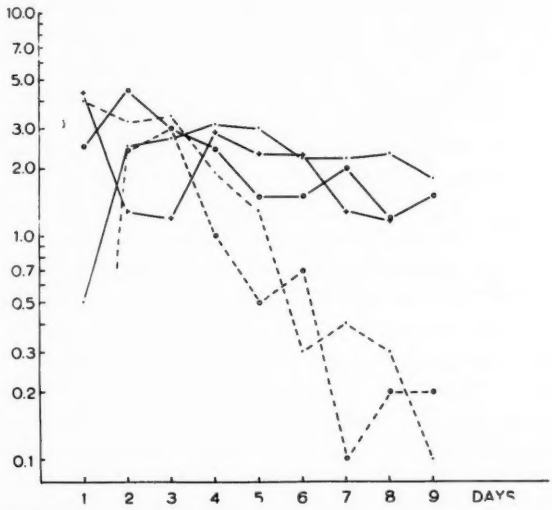

Fig. 14. Average daily P^{32} mating loss, in per cent of Day 0 activity. Dashed curves indicate ♂♂ kept on yeast, while full curves show ♂♂ on honey. Semilog scale. ●=LXVI. ○=LXXV. +=XCVI.

non-mated flies, a curve showing the mating loss will be obtained, expressed in per cent of the Day 0 activity. The derivative with respect to time of this curve will show the rate with which this difference increases during the experimental period. In the present material the derivation has been done graphically on the basis of the smoothed retention curves. The results are presented in Fig. 14, which shows the mating loss per day in ♂♂ on honey and on yeast diets. It will be noted that over the first 9 days, on honey the mated ♂♂ lose about 2 per cent per day more than do non-mated ♂♂, and the ♂♂ on yeast show the same difference in rate of loss on the first 3—4 days, but this differ-

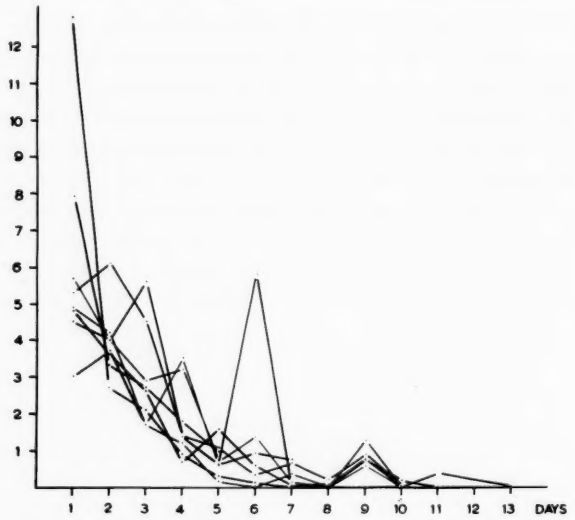

Fig. 15. Amount of P^{32} transferred from ♂♂ kept on yeast to ♀♀ through mating on different days after ingestion, expressed as pro mille of ♂'s original P^{32} content. (XCVI.)

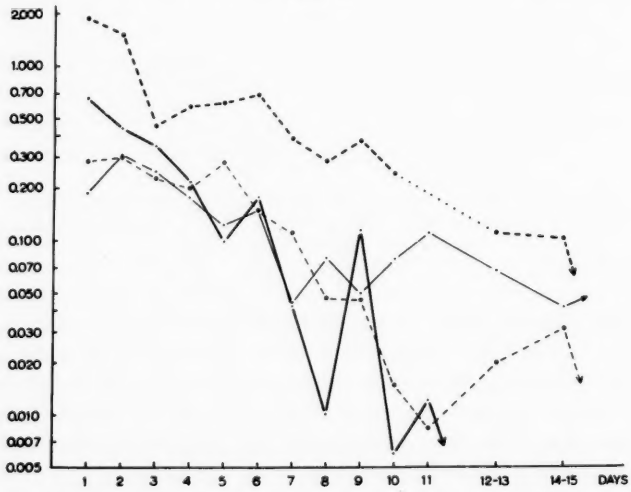

Fig. 16. Average productivity and average mating transfer of P^{32} of ♂♂ kept on honey and on yeast (XCVI). Semilog scale.

Thin dashed line: Offspring in honey group. Heavy dashed line: Mating transfer in honey group. Thin full line: Offspring in yeast group. Heavy full line: Mating transfer in yeast group. Ordinate: For offspring production: Number of offspring $\times 10^{-3}$. For mating transfer: Activity of ♀♀ in per cent of ♂'s Day 0 activity.

ence decreases to one tenth in the course of the next 4—5 days. It is therefore obvious that the difference in P^{32} content between mated and non-mated ♂♂ is built up on yeast in the course of a few days, and then at a sharply declining rate, while on honey it grows over a longer period at a nearly constant rate.

The radioactivity contents of corresponding groups of ♀♀ have been measured at the end of the mating period. If expressed as fraction of the ♂'s Day 0 activity, the measurement averages give the same results as the comparisons between the mating losses in the ♂♂, namely a sustained transfer on the honey diet, and an initially high but rapidly decreasing transfer on yeast. The variation in P^{32} content of the ♀♀ is high. An example is given in Fig. 15, showing the values of the different groups of ♀♀ mated to individual ♂♂ on yeast in Experiment XCVI. Other experiments show essentially the same degree of variation.

The mating transfer of radioactivity may be compared with the productivity in the same ♂♂. This has been done in Fig. 16.

In Figs. 17 and 18 have been compared the shapes of the curves of the daily averages of mating loss, mating transfer, and offspring production in groups on honey and on yeast in XCVI. It will be noted that there is a better agreement between the shapes of the three curves in the honey group than in the yeast group. In the former (Fig. 17) there is a similarity of slopes for the first six or seven days, after which the productivity falls off to low values. On yeast (Fig. 18), on the contrary, it is the offspring curve which falls off less, while mating loss and mating transfer fall off more rapidly.

The productivity figures discussed above should be corrected for the occurrence of dominant lethals (Table 7). Data are not available for making such corrections with differences in retention on honey and on yeast diet taken into consideration. However, it is expected that such corrections would lead to little change in the honey group comparison, but to greater divergence of the yeast group curves.

These conclusions can be checked by observing the correlation between productivity and radioactivity content in different groups of ♀♀. The results for Experiment XCVI are given in Table 6 in terms of regression and correlation coefficients. From the table it is obvious that the results are highly variable, and no clear cut tendency may be read out of them. However, the correlation between radioactivity transfer and offspring production is slightly higher in the honey group than in the yeast group. Also, all regressions in the honey group are positive, while in the yeast group they are negative on several days.

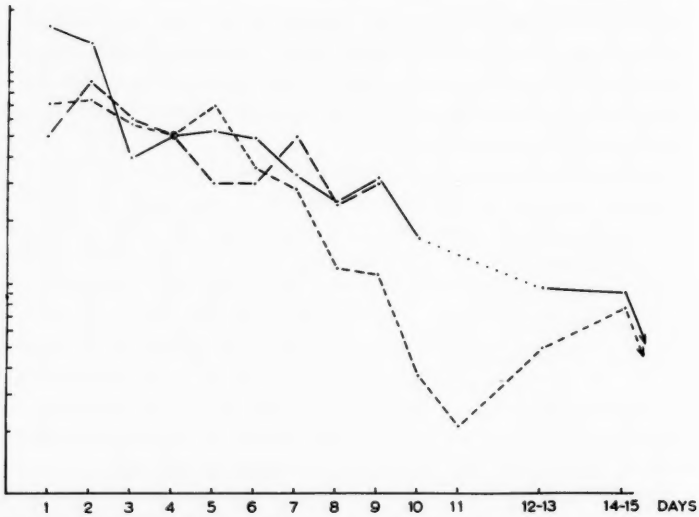

Fig. 17. Comparison of daily mating loss in ♂♂, mating transfer to ♀♀, and offspring produced, in flies kept on honey (XCVI). The curves have been arbitrarily normalized to coincide on Day 4. Semilog scale.

Long dashes: mating loss. Full line: mating transfer. Short dashes: offspring produced.

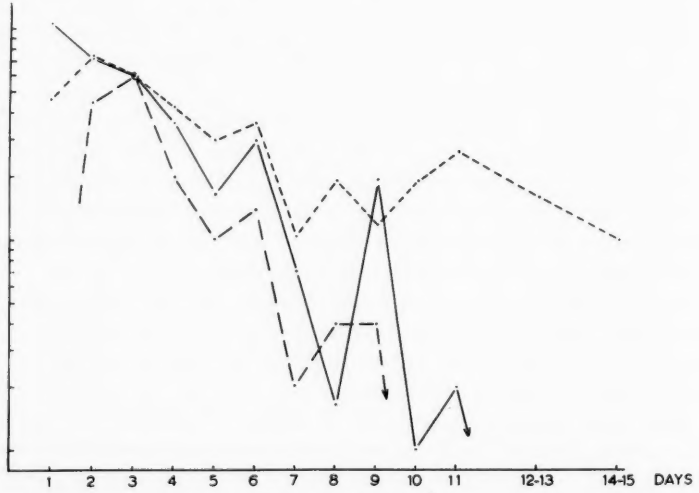

Fig. 18. Comparison of daily mating loss in ♂♂, mating transfer to ♀♀, and offspring produced, in flies kept on yeast (XCVI). The curves have been arbitrarily normalized to coincide on Day 3. Semilog scale.

Long dashes: mating loss. Full line: mating transfer. Short dashes: offspring produced.

TABLE 6. *Relations between mating transfer of P^{32} and offspring production (XCVI).*

Mating period	Honey mated		Yeast mated	
	r	b	r	b
1	0.620	0.0604	0.353	0.0102
2	0.464	0.0152	0.686	— 0.0102
3	0.459	0.00691	0.120	— 0.000939
4	0.241	0.00268	0.439	0.00220
5	0.573	0.0107	0.114	0.000444
6	0.345	0.00954	0.173	0.000757
7	0.310	0.00489	0.171	0.000827
8	0.191	0.00564	0.221	0.00201
9	0.515	0.0311	0.630	— 0.00952
10	0.370	0.0158	0.192	0.000397
11	no data		0.540	— 0.00115
12—13	0.409	0.00489	0.0	
14—15	0.284	0.00274	0.0	

$$r_{x_1x_2} = \frac{\Sigma(x_1-\bar{x}_1)(x_2-\bar{x}_2)}{\sqrt{\Sigma(x_1-\bar{x}_1)^2(x_2-\bar{x}_2)^2}} \qquad b_{x_2/x_1} = \frac{\Sigma(x_1-\bar{x}_1)(x_2-\bar{x}_2)}{\Sigma(x_1-\bar{x}_1)^2}$$

where *r* is the correlation coefficient between offspring production (x_1) and mating transfer (x_2), and *b* is the regression coefficient for mating transfer on offspring.

x_1=total number of offspring produced by all ♀♀ mated to one ♂ for one mating period. x_2=radioactivity content of all ♀♀ mated to one ♂ for one mating period, measured at the end of the mating period and expressed as pro mille of the ♂'s initial radioactivity content.

The finding of a better correlation in the honey group is easily understood, because it is to be expected that after the first few days sperm and seminal fluid will be tagged to a higher degree, since there is little renewal of the phosphorus pool. The same aspect explains why the regression coefficient b is at all times higher in the honey than in the yeast group, indicating higher relative P^{32} transfer per offspring.

4. The shift in P^{32} distribution

The curves in Figs. 2 and 3 show the retention in the fly as a whole. As has been calculated by KING (1952) the P^{32} in the head and thorax contribute very little — about 1/70 — to the total radiation dose received

by the testes. In homogeneously P^{32} labeled flies, KING (1954 b) finds about 62.8 per cent of the radioactivity of the fly contained in the head, thorax, wings, legs, and ventriculus; the remaining 37.2 per cent are contained in the different structures in the abdomen (20.3 per cent) and in the haemolymph (16.9 per cent). On the other hand, in flies that have been allowed to feed for only 24 hours on radioactive medium, KING (1952) finds 57 per cent in the abdomen.

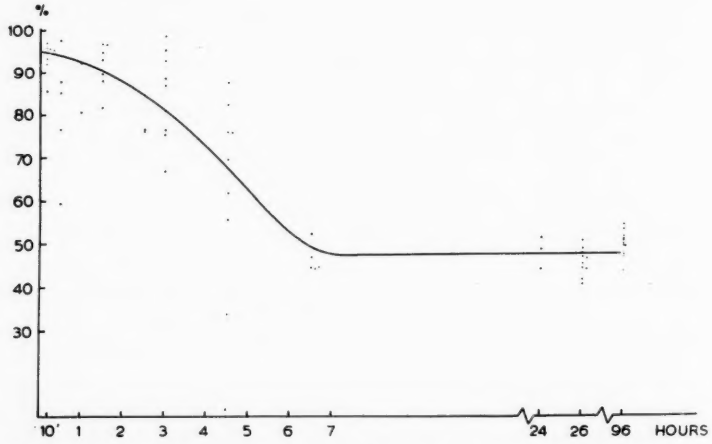

Fig. 19. Fraction of total ingested P^{32} contained in the posterior half of the fly, determined by bisection. Dots indicate individual measurements. The line has been drawn by eye. Initial P^{32} content not measured (CXIV—CXVII).

In order to evaluate the dose absorbed by the genetic material in the present experiments, the retention curve for the abdomen relative to the head and thorax has been determined: CS+ ♂♂ less than 3 hours old were given a 10 minute meal of P^{32}. At different intervals after feeding males were etherized, frozen hard with CO_2-ice, and while still frozen bisected along a plane running vertically just posterior to the scutellum and between the bases of the second and third pairs of legs. The operation is performed quickly, with a cooled scalpel. Before thawing has occurred, the two parts of the fly are put separately into gelatin capsules, and their radioactivities measured. In this way, the crop is not perforated, and is completely included in the posterior part, even when distended shortly after a meal. A small part of the muscle mass

of the thorax will also be included in the posterior part. In Fig. 19 are shown the combined results from several experiments. The fraction of the total radioactivity contained in the abdomen is plotted against time. It will be seen that a constant distribution at this level of dissection has established itself after about 7 hours. The abdomen then contains about 47.5 per cent of the total radioactivity. The average weights of the two parts of the fly have been determined in another similarly fed and bisected group, and proved to be about 60 per cent for the head-thorax-wings part, and 40 per cent for the abdomen, showing that the distribution attained in 7 hours approaches homogeneity by weight. The difference observed between the present results and KING's (1952) 24 hour feeding results may possibly be taken to indicate that the gut and crop under KING's conditions of feeding contain a higher amount of radioactivity, possibly bound to undigested organic compounds from the food.

5. Conclusions from retention data

The curve for the retention of radioactive phosphorus in a *Drosophila* ♂ after a single meal of the radioisotope may be formally described as the sum of three exponential functions. The exponent of each of these functions — or the rate of each of the processes described by the function — is influenced by the diet on which the fly is kept. The rates are also influenced by mating if the food is yeast, which is relatively rich in phosphorus. On honey, where the flies are on a phosphorus deficient diet, the rate of the slowest of the processes appears not to be influenced by mating (Fig. 10). — The differences in shape of the retention curves may also be taken to indicate shifts in the relative importance of the different processes. More P^{32} is excreted by way of the medium slow process in mated ♂♂ than in non-mated ♂♂, and the same holds for ♂♂ on yeast in comparison with ♂♂ on honey (Fig. 9). The over-all differences between groups are, however, complex and cannot be ascribed to changes in rate or importance of any single process.

The ratio of the retentions in groups on yeast and on honey decreases — after the first 2—3 days — in an essentially exponential way (Fig. 11). This description shows a better fit if the comparison is made between non-mated groups than if between mated groups. — The ratio of the retentions in mated and non-mated groups on the same diet shows essentially an exponential relationship for groups on yeast for Days

1—9. For groups on honey, the expected exponential relationship is not apparent. The reason for this result is not known.

The differences in P^{32} content between mated and non-mated groups on the same diet — expressed as per cent of the average Day 0 radioisotope content — develop at a sharply decreasing rate in flies kept on yeast, and at a slowly decreasing rate in flies kept on honey (Fig. 14). This mating loss curve has the same general shape as the corresponding curve for mating transfer of P^{32} to ♀♀ (Figs. 17, 18). Also, the mating transfer shows a higher correlation with the number of offspring produced in the honey group than in the yeast group. However, the correlation is at no time strikingly high, which indicates that the most important pathway of P^{32} transfer is the seminal fluid, and not the sperm.

Measurements of ♂♂ shortly after a brief meal of P^{32} reveal no inflection in the retention curve, indicating a rapid entry of the P^{32} into the metabolism of the ♂♂ (Fig. 3).

Transverse bisection of ♂♂ at different times after a brief meal indicates that a stable distribution of P^{32} is attained about 7 hours after ingestion (Fig. 19).

C. P^{32} incorporation in sperm

1. Introduction

The incorporation of P^{32} into biological material has been used to map the turnover and synthesis of nucleic acids, both RNA and DNA (HEVESY and OTTESEN 1943). The present experiments have been planned with the studies of HOWARD and PELC (1951 a, b) in mind. These authors show how synthesis of DNA takes place only in the proliferating part of the bean root, while the cells undergoing differentiation but not mitosis do not show DNA synthesis. According to their technique, DNA-phosphorus may be estimated after a 10′ hydrolysis in 60° 1 N HCl. HOWARD and PELC use radioautographs to demonstrate the presence of radioactive material. The same method was used by PLAUT (1953), also on plant material, and by ORTAVANT (1956) on testicular material from ram.

TAYLOR and MCMASTER (1954) have published a very careful study of the reliability of the acid hydrolysis method in studies of P^{32} incorporation into DNA in *Lilium*, comparing it with enzymatic digestion. According to their results, acid hydrolysis is the less sensitive method insofar as it removes some P^{32} which is not affected by DNAase. In both methods, there remains some P^{32} which is not removed by hot trichlor-

acetic acid, and which presumably represents incorporation into phosphoproteins. In view of the simplicity of the technique, acid hydrolysis appears to be the better method with the present material, possibly also in others (PARTANEN and GIFFORD 1958).

In *Drosophila* it is difficult to differentiate all stages of spermatogenesis under the microscope, the cells being small, and the stages are not easily separated when maturing spermatids are to be studied. Radioautographs of mixed cell populations are therefore of limited use. A description of spermatogenesis in *Drosophila*, based on work with ordinary cytological methods, is given by COOPER (1950). In the present study, only one stage has been used, namely mature sperm, and this stage has been separated from the others by collecting sperm in mated females. Assay of the amount of P^{32} in the sperm has been made with the counter assembly described earlier.

2. Preliminary experiments

In preliminary experiments the hydrolysis technique of HOWARD and PELC was adapted to the new material, by testing different hydrolysis times and temperatures on *Drosophila* testes. In all tests 1 N HCl was used. It was found that after 10—12 minutes hydrolysis at 60° C, a stable level of activity remained in the testes if the activity were measured in per cent of the ♂'s original activity, and that this was constant

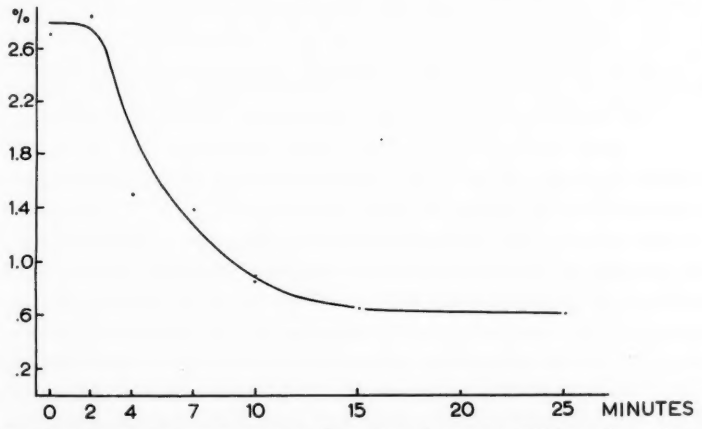

Fig. 20. Cpm in the testes of unmated flies three days after P^{32} ingestion, after different hydrolysis times, expressed as per cent of c.p.m. in the male prior to dissection. Counter no calibrated, so absolute values cannot be given. (Experiment X.)

up to 25′ of hydrolysis (Fig. 20). With hydrolysis at 100° C, no such stable niveau was found. Accordingly, it was decided to use a 15 minutes hydrolysis at 60° for the routine measurements.

At the same time, it was found that the remaining radioactivity after hydrolysis of testes from non-mated males was reasonably constant for each day after feeding when expressed as fraction of the radioactivity of the living male prior to dissection. This remaining radioactivity

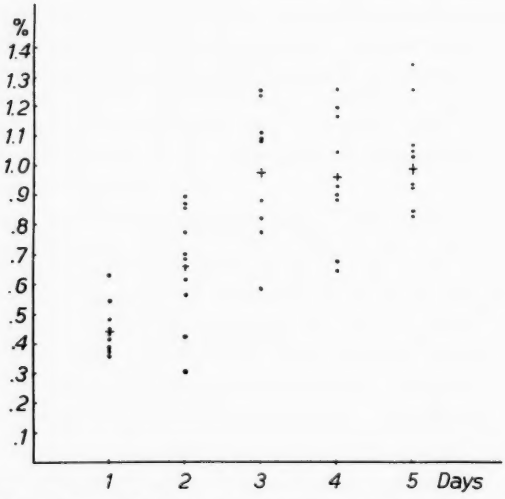

Fig. 21. Cpm from P^{32} remaining in the testes of unmated ♂♂ after hydrolysis, expressed as per cent of the c.p.m. of the male prior to dissection. Counter not calibrated, so absolute amounts cannot be given. + = mean value.

reached a level of about 1 per cent on the third day after feeding, and remained so on the following days (Fig. 21).

Several factors are involved in determining the measured level of incorporation of P^{32} into the DNA in the testes. As shown later, the bulk of the tagged sperm will be mature on Day 7—8, and since productivity measurements show a minimum on Days 9—10, the number of spermatocytes synthesizing DNA probably falls off rather rapidly during the first day or two of imaginal life. Also, some Day 1 sperm, which become most heavily "contaminated" (see Figs. 25, 26), will in the course of 2—4 days degenerate in unmated ♂♂, and their contribution to the radioactivity of the testes will disappear.

The holding back of mature sperm by unmated ♂♂ has been demonstrated by MOSSIGE (1955), and is the reason for the use of as many as five ♀♀ per ♂ per day in the present experiments. Other authors (LÜNING 1952, BATEMAN 1956) have used less exhaustive mating patterns, maintaining that mature sperm is resorbed so rapidly that it has no blurring effect on the mutation pattern studied. How blurring is avoided in BATEMAN's experiments is not easily understood, since it is stated that the ♂♂ are capable of mating with more ♀♀ than provided per day, and that old sperm are used up only after 3—4 days' mating.

3. Sperm degeneration

Some incidental observations in the present material throw some light on the process of sperm degeneration in ♂♂.

In the preliminary experiments, the P^{32} incorporation in the testes was compared with that in the accessory glands. A lower level of incorporation was found in this organ, but only by a factor of about 10. At first the reason for this latter radioactivity was not understood, since there should be very little non-hydrolyzable phosphorus in these glands. However, later it has been discovered that in non-mated males, the glands — which are hollow sacs — become packed with debris, most of which appears to be degenerating sperm. Fig. 22 shows phase contrast photographs of an accessory gland dissected from an unmated 3 day old ♂. The sperm tails are a quite prominent feature of the gland's contents, as is the large number of spindle shaped bodies which may be sperm heads in different stages of degeneration or autolysis. Sperm degeneration therefore apparently takes place in these organs, a function which was not suspected earlier. (For discussion of anatomy and function of the glands see MILLER, 1950.) It is at present not clear to what extent the spindle-shaped bodies in the accessory glands represent degenerating sperm. Thus, they may be seen in the gland in < 12 hour old ♂♂, where the sperm have not yet descended into the seminal vesicles. Also, the Feulgen reaction is absent after 15′ hydrolysis, but since this is not the optimum time this observation is equivocal. If the capacity for Feulgen reaction is rapidly diminished during karyolysis, it may be difficult to demonstrate the sperm heads by this method.

Because there is such a pronounced difference between the total retention in mated and non-mated ♂♂, it seems probable that a resorbtion of the material from the lysed sperm takes place. If the sperm were ejaculated without mating, or the lysate excreted, no difference

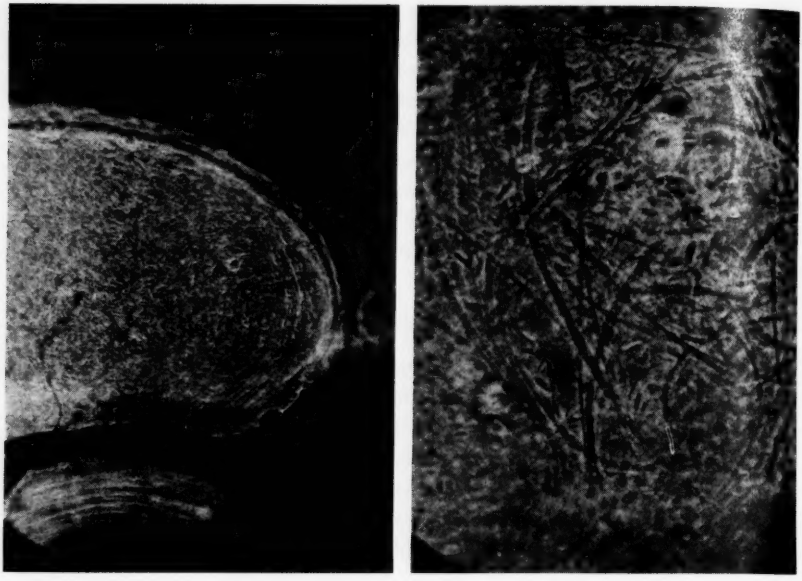

Fig. 22. Phase contrast microphotographs of testis and accessory gland of 3-day old unmated *Drosophila* ♂.

Left photo: Seminal vesicle of testis (below) packed with sperm, and accessory gland (center) packed with spindle shaped bodies and sperm tails (×44). Right photo: Accessory gland at higher magnification. This gland contained more sperm tails than have usually been seen (×105).

from mated ♂♂ would be expected, since the P^{32} in both mated and non-mated ♂♂ would leave the pool after incorporation into sperm. The present results indicate that this exit from the phosphorus pool is at least partly closed in the non-mated ♂♂, and that presumably a major part of the difference between the two groups of ♂♂ may be explained by this factor, making it unnecessary to seek other reasons, e.g. changes in metabolism caused by greater sexual activity.

Sperm tails have been found in accessory glands in unmated ♂♂ within 24 hours of eclosion. Sperm degeneration therefore begins quite early. Since the rate of sperm degeneration is not known, nor the rate of production, it is impossible to say how long a period the mature sperm in the seminal vesicle of unmated ♂♂ represent.

4. Variables to be considered

The radioactivity measurements of the hydrolyzed sperm from a group of ♂♂ provide an estimate of the P^{32} content in the total sperm production in one day. In order to give meaningful results in terms of P^{32} content per sperm, it is also necessary to have an estimate of the number of sperm produced per male per day. This estimate may be based on offspring production per male per day obtained from other groups of males, but will be subject to several modifications, such as polyspermy, dominant lethal effects of the radiation from P^{32} in the fly, and culture conditions. The calculations have been summarized in the formula below, the different factors of which will be discussed further:

$$\text{n } P^{32} \text{ atoms per sperm} =$$

$$= \frac{\text{cpm in hydrolyzed sperm}}{\text{number of sperm}} \times \frac{\text{atoms per C } P^{32}}{\text{disint. per C } P^{32}} \times \text{geom. fact.}$$

$$= \frac{\text{cpm in hydrolyzed sperm}}{\text{offspring} \times \text{polyspermy} \times \text{dominant lethal factor}} \times$$

$$\times \frac{6.599 \times 10^{16}}{2.22 \times 10^{12}} \times \frac{100}{34.76}$$

Taking the polyspermy factor first, according to KAUFMANN and DEMEREC (1942) each offspring represents a sperm consumption of between 20 and 30, although actual polyspermy involves only about 8 sperm per fertilization. For the present, the figure of 25 sperm per offspring is used, and this number is assumed to be constant during the experimental period.

The maximum number of sperm produced per ♂ per day will — according to the present data — be of the order of 7500 on Days 2—4 (300 offspring and 25 sperm per offspring). This figure does not appear improbable in view of the observations of KAUFMANN and DEMEREC (1942) who counted a maximum of about ten thousand sperm ejaculated from one ♂ in four matings.

Turning next to the offspring production figures, several ways of obtaining an estimate may be proposed. In earlier calculations (OFTEDAL and MOSSIGE 1957 a, b) the figures provided by the parallel genetic experiment have been used, with no further corrections. In contrast, offspring production figures from an ordinary control experiment will give an estimate of the sperm production under no radiation load. If the effectivity of the sperm is affected by radiation by way of dominant

lethals, but not the processes of spermiogenesis, the unirradiated control should give the best value for the number of sperm incorporating P^{32}. If, however, the dominant lethal effects are of minor or similar importance in comparison with variations due to uncontrolled experimental conditions, the simultaneous parallel experiment should give the best comparison.

Brood patterns for dominant lethals have been obtained by several authors (BAKER and SGOURAKIS 1950, LÜNING 1952, BATEMAN 1956, 1957 for *D. melanogaster*, ALEXANDER and STONE 1955 for *D. virilis*), but only after acute irradiations. BATEMAN's data are from experiments where a brood technique similar to the present one has been used. They show that there is a tenfold increase in sensitivity between mature sperm and meiotic spermatocytes at the dose level of 1000 r, but give no information on the dose-effect curve for any stages. Also, BATEMAN interprets his data on egg laying to indicate that the fertility — in this case meaning sperm production — is essentially unchanged by irradiation. However, he bases this assumption on only one day's laying from each brace of ♀♀ used in mating, and this hardly gives a complete picture of the total amount of sperm transferred per day. Therefore, the literature appears not to contain sufficient data for determination of the fertility reduction and the dominant lethal frequency in the present material.

The *total* offspring production per ♂ over an 8—10 day period in several control, and acute or chronic irradiation experiments give little indication of a predominant radiation effect on the fertility of the ♂♂. Since, however, the total figure is of less interest than the day to day variation, particularly on Days 5 to 8, the shape of the offspring production curve is more important. Comparison between curves from controls, acute irradiation experiments, and chronic irradiation experiments shows that while there is a sterility period on Days 9 and 10 after acute exposure, this is not as pronounced after chronic irradiation, though production is reduced after Day 8 even in the non-irradiated controls. Furthermore, the radiation load in isotope experiments is not so heavy that it covers the variation to be seen between control experiments with no radiation load. On the other hand, in parallel experiments performed at the same time, it may be seen that ♂♂ containing P^{32} give fewer offspring than their non-radioactive controls and also that flies with a lower average P^{32} content produce more offspring than parallel groups with higher P^{32} content. The differences involved are, however, all within the variation found in experiments with non-irra-

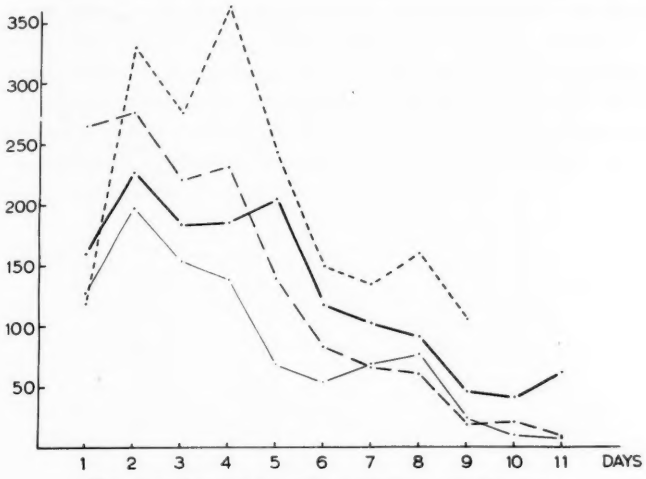

Fig. 23. Offspring per day in different experiments.
Heavy full line: No P^{32} content (XXIV). Thin full line: P^{32} content 0.049—0.032 μC (XXIV). Long dashes: No P^{32} content (XX). Short dashes: No P^{32} content (XXXII).

diated flies. Fig. 23 shows the results of one simultaneous P^{32} and control experiment (XXIV), together with those from two other control experiments. The actual figures for the two former are presented in Table 7. The radiation load in this P^{32} experiment (XXIV) is somewhat lower than those of the incorporation experiments XXIII and XLI, and it would therefore be expected that the depressive effect on productivity seen in XXIV should be at least equalled in the incorporation experiments, perhaps with the exception of the Day 5 factor, which appears to be spuriously high.

We are therefore faced with three sets of data on which to base the estimate of the sperm production per male per day in the incorporation experiments: namely, unirradiated control experiments performed at other times, *or* parallel experiments with similar radiation loads, *or* the latter data qualified by means of a third set of data from P^{32} and control experiments performed simultaneously but at different times from the incorporation experiments.

The choice between these different ways of calculation is not altogether easy. The use of unqualified control material seems less attractive, since variation between experiments appears to be of the same magnitude as the radiation effects one tries to compensate for. The use

TABLE 7. *Offspring per ♂ per day in control and P^{32} groups in Exp. XXIV.*

P^{32} content per ♂ 0.049—0.032 μC, n ♂♂ in controlgroup=10, in P^{32} group=10.

Day	Control	P^{32} group	$\frac{\text{Control}}{P^{32}}$	Calculated n sperm in control
1	159	127	1.25	3975
2	225	197	1.14	5625
3	182	153	1.19	4550
4	184	136	1.35	4600
5	203	67	3.06	5075
6	116	52	2.23	2900
7	101	67	1.51	2525
8	90	75	1.20	2250
9	44	23	1.91	1100

of the production data from the parallel genetic experiments certainly appears better. Since culture conditions are of as great importance as they obviously are, simultaneity of the experiments is highly desirable, but the disregard of dominant radiation effects may cause occlusion of some aspects, overemphasis of others. The introduction of correction factors to the latter data based on parallel experiments with irradiated ♂♂ takes account of the radiation load and appears to be the best solution.

However, the use of non-irradiated controls in the calculations, either directly or as a basis for corrections, necessitates the assumption that the predominant radiation effect during spermiogenesis is on the genetic level and not on the physiologic. That is to say, the number of sperm produced per day is not influenced by the radiation load. This assumption is probably fully justified for the first two or three days, but becomes increasingly uncertain as the time passes and the importance of the assumption increases. It has been assumed by BATEMAN (1956) that the variation in fertility in his experiments is due solely to dominant lethality. This may be so in his material, but it is less certain with the present experimental setup — where the total number of sperm is important, not only the relative frequency of dominant lethals.

It has recently been shown by EDWARDS and SIRLIN (1958) that in the mouse 200 r induces pronounced temporary oligospermy, but no change in the rate of spermatogenesis or spermiogenesis. FRIESEN (1937) and WELSHONS and RUSSELL (1957) found degenerative changes in the

regions of the *Drosophila* testis where young spermatocytes and secondary spermatogonia are usually seen, after acute doses of 1000 r and 4000 r. The doses absorbed in the testes during the first 24 hours of the present experiments do not appear to be higher than about 500 rep. Unless chronic irradiation is much more effective than acute in killing these cells, one would therefore not expect cell death to be very important in the present case.

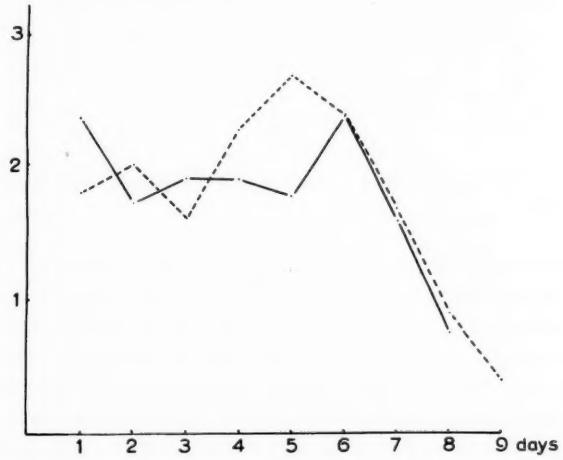

Fig. 24. Average P^{32} activity remaining in ♀♀ after hydrolysis. Ordinate $= \frac{\text{cpm}}{\text{n } ♂♂}$. Full line: Exp. XXIII. Dotted line: Exp. XLI.

5. Experimental results and discussion

In the experiments on the incorporation of P^{32}, the males have been mated as described earlier. At the end of each mating period, the females from all the males in the group have been collected and the vagina with the attached seminal receptacles cut off. Usually no special efforts have been made to remove the rectum or adhering fat tissues. The whole of the dissected tissues have then been fixed in acetic alcohol 3 : 1 for 1—2 hours, and then taken through the alcohols to water. After hydrolysis in 60° 1 N HCl for 15 minutes they have been washed in three changes of 2 per cent secondary sodium phosphate, taken through the alcohols to absolute, and then mounted on a lead plaquette by means of one drop of a very thin solution of Euparal for assay of radioactivity.

The raw data from two incorporation experiments, with which cor-

TABLE 8. *n P^{32} atoms per sperm in Exp. XXIII.*

Day	$\frac{\text{cpm}}{\text{n } ♂♂}$	$\frac{\text{offspring}}{\text{n } ♂♂}$	n sperm = corr. offspr. × 25	n atoms per sperm	n atoms per sperm acc. to XXIV control
1	2.36	123.8	3867	51.1	50.8
2	1.72	246.9	7037	20.9	26.3
3	1.90	321.4	9401	17.3	35.7
4	1.89	160.7	5425	29.8	35.1
5	1.77	130.9	10014	15.1	29.8
6	2.37	99.9	5402	37.5	71.7
7	1.59	26.8	1012	134.4	53.9
8	0.75	21.0	630	101.7	28.5
9	0.22	5.4	258	72.9	17.1

Average number of P^{32} atoms per sperm for XXIII Days 2—6 and XLI Days 2—6 (Table 9) = 22.8.

responding genetic experiments were performed at the same time under identical conditions, are presented in Fig. 24. The relevant data for offspring production in Exp. XXIV are presented in Table 7, together with the factor for dominant lethal effect to be used in subsequent calculations, and with the calculated sperm number per male per day. It may be seen that the dominant lethal effect is highest on Days 5—6. If the data are plotted in terms of dominant lethal frequency, the shape of the curve up to Day 5 is rather similar to that found by BATEMAN (1956). However, while BATEMAN finds a plateau for Days 5—8, in the present material there is a peak on Day 5, with subsequent decline. This differ-

TABLE 9. *n P^{32} atoms per sperm in Exp. XLI.*

Day	$\frac{\text{cpm}}{\text{n } ♂♂}$	$\frac{\text{offspring}}{\text{n } ♂♂}$	n sperm = corr. offspr. × 25	n atoms per sperm	n atoms per sperm acc. to XXIV control
1	1.79	116.3	3633	42.1	38.5
2	2.00	311.3	8872	19.3	30.5
3	1.60	294.5	8761	15.6	30.1
4	2.27	229.2	7736	25.1	42.2
5	2.67	235.6	18023	12.7	45.0
6	2.38	146.4	8161	24.9	72.0
7	1.68	88.6	3344	43.0	56.9
8	0.90	38.6	1158	66.4	34.2
9	0.39	12.5	597	55.9	30.4

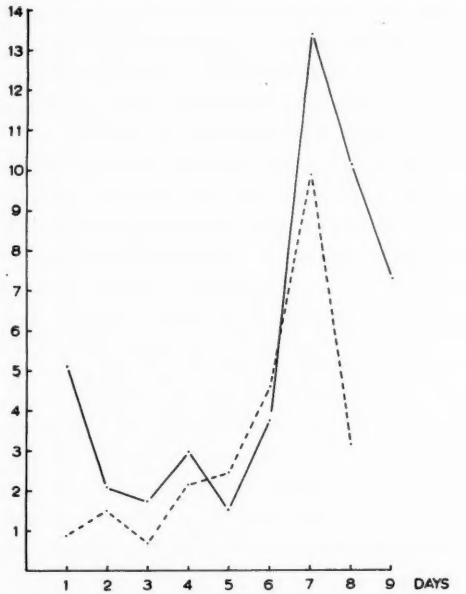

Fig. 25. Incorporation and genetic effect (XXIII).
Full line shows number of P^{32} atoms per sperm remaining after hydrolysis times 10^{-1}. Dotted line shows number of sex-linked lethals in per cent.

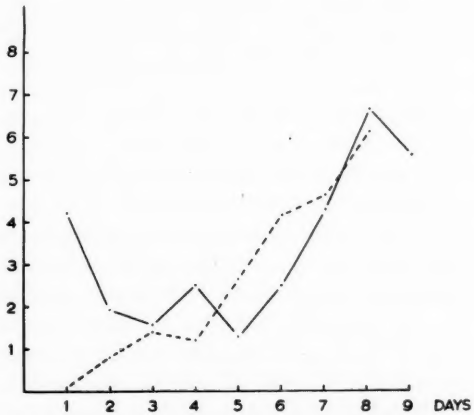

Fig. 26. Incorporation and genetic effect (XLI).
Full line shows number of P^{32} atoms per sperm remaining after hydrolysis times 10^{-1}. Dotted line shows number of sex-linked lethals in per cent.

ence may be caused by BATEMAN's use of only two ♀♀ per ♂ per day, which would be expected to cause a broadening of the peak.

In the fifth column of Table 7 will be found the estimated number of sperm per day per ♂ in the control group. Exp. XXIV did not give high offspring numbers, and it will be seen that the figures for sperm production therefore are lower than in other experiments.

The incorporation data from two experiments (XXIII and XLI) are presented in Tables 8 and 9. The second and third columns give the measurements of incorporated P^{32} corrected for number of ♂♂, and the offspring production in the genetic group for each day. In the fourth column the sperm number is given, calculated from offspring production, dominant lethal effect factor from Table 7, and a consumption of 25 sperm per offspring. It will be seen that the calculated sperm number only once (XLI-Day 5) exceeds the maximum mentioned above from KAUFMANN and DEMEREC (1942). In the fifth column are presented the incorporation data, in terms of P^{32} atoms per sperm. These figures are presented graphically in Figs. 25 and 26, together with the genetic results. The incorporation data and the genetic results have been used earlier in brief presentations of the relative values (OFTEDAL and MOSSIGE 1957 a, b).

The incorporation per sperm is about 15 to 30 radioactive atoms per sperm for Days 2—5, after which there is a sudden increase to peak values of 134 and 66 on Days 7 and 8 in the two experiments. The peak incorporation coincides with the peak genetic effect, and both the shift in time and the difference in shape of the genetic curve between experiments appears to be paralleled in the incorporation curve. It seems plausible that the incorporation on Days 2—5 represents a contamination of P^{32} in sites other than DNA, probably in proteins (TAYLOR and MCMASTER 1954, HEATH, RIMINGTON and MANN 1957). This is also a natural explanation for the higher count found on Day 1, when the P^{32} content of both fly and seminal fluid is at its highest.

Turning again to the other methods for estimating the number of sperm and thereby incorporation, it will be seen from earlier reports (OFTEDAL and MOSSIGE 1957 a, b) that the use of the unqualified production data from the parallel genetic group of ♂♂ gives a very close correlation between P^{32} incorporation and mutation frequency. According to the arguments above, and as will be further discussed below, this apparent relationship is spurious and not meaningful.

Finally, the incorporation data calculated on the basis of an unrelated, unqualified control experiment (the untreated group in Exp.

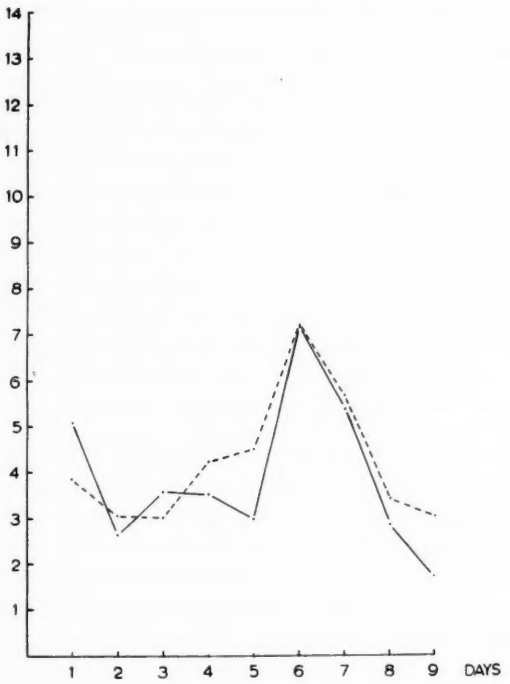

Fig. 27. Incorporation when sperm number is calculated on the basis of unrelated controls, showing number of P^{32} atoms per sperm remaining after hydrolysis times 10^{-1}. Full line: Exp. XXIII. Dotted line: Exp. XLI.

XXIV, Fig. 23) are presented in Fig. 27. It will be noticed that in both experiments the peak is of the same magnitude and occurs on Day 6. Thus, the conformity between the incorporation experiments appears close, while the relationship with the genetic experiments is not apparent. Apart from the arguments presented earlier, the results obtained by this method are therefore less appealing, since the differences known to exist between Exps. XXIII and XLI in initial P^{32} content of the ♂♂, productivity, sex-linked lethal frequency, and even experimental setup, are apparently not reflected at all in the incorporation data. Furthermore, the results presented in this form cannot be interpreted to provide a useful working hypothesis on which further studies may be based.

6. Conclusions

The present data show that in young *Drosophila* ♂♂ the period between the last DNA synthesis in spermatocytes and the transfer of mature sperm derived from these cells, at the most is eight days, and probably not shorter than six, as indicated by P^{32} incorporation. Together with the genetic data obtained with the present brood system, which show that the last radiation induced translocations — and the first induced crossovers — occur on Days 6—7 (FRIESEN 1937, MOSSIGE 1958, KVELLAND, personal communication), the present findings therefore seem to indicate that the last DNA synthesis in the male germ line is completed very shortly before the first meiotic division.

The length of the synthesis period cannot be determined from the present type of experiment, where the availability of P^{32} for the DNA synthesis is not constant, and the sampling of cells is made only at 24 hour periods. The question could probably be answered by using a constant load of tracer, and determining the period elapsed between onset of tagging and the attainment of maximal labelling, the most suitable technique being quantitative autoradiography of immature testes.

These results invalidate the earlier tentative conclusions (OFTEDAL and MOSSIGE 1957 a, b) that incorporation and mutagenesis were closely correlated for the whole observation period, and that this might indicate either a causal relationship through the mutagenic effect of the change from P^{32} to S^{32} in DNA, or a coincidence in time between metabolism and radiosensitivity. The question is yet unanswered regarding the relative importance of radiation effect and transmutation effect on Day 7 and later, when incorporation into DNA has taken place. A discussion of this aspect follows below, but first it is necessary to discuss the radiation effects to be expected in some detail.

III. STUDIES ON THE ISOTOPE RADIATION EFFECT

The physical constants of the two radioisotopes used in the present study — phosphorus-32 and yttrium-91 — are as follows:

	P^{32}	Y^{91}
Type of radiation	pure β	pure β
Maximum energy	1.71 MeV	1.52 MeV
Average energy	0.69 MeV	0.623 MeV
Half life	14.3 days	61 days
Range in water	~7.5 mm	~6.5 mm

A. Radiogenetic effect of the retained P^{32}

1. Genetic results

In two experiments (XXIII and XLI) the genetic effects of P^{32} ingestion have been scored in parallel with the amounts of P^{32} incorporated into sperm (OFTEDAL and MOSSIGE 1957 a, b). The effects have been measured by means of sex-linked recessive lethals, obtained in brood pattern after providing the ♂♂ with five virgin M-5 ♀♀ per day. The mutation frequencies observed are shown in Figs. 25 and 26. It will be noted that the germ cell sensitivity pattern found after P^{32} ingestion is somewhat similar to the one observed after acute irradiation (Fig. 34), but that the peak is delayed, and that the increase in sensitivity with time is somewhat more gradual.

2. Factors to be considered

In trying to interpret the quantitative differences observed between the effects of different radiation treatments, the determination of the genetically significant dose will depend directly upon the relative biological efficiency ascribed to the radiation in question, and upon the fraction of the total dose presumed to be absorbed in the tested. A discussion of these two factors therefore follows.

a. *The relative biological efficiency (RBE)*

The relative biological efficiency of β-particles has been the subject of numerous investigations (see review by BOAG 1953). Because of the more widespread use of β-emitting radioisotopes both for tracers and for radiation sources the question has gained in importance.

The early studies by ZIMMER, GRIFFITH and TIMOFÉEFF-RESSOVSKY (1937) led to the conclusion that the RBE of radium β-particles is 1.0 in comparison with X-rays. These authors measured the effect of the radiations by means of recessive lethals in *Drosophila.*

Other authors have used different materials, and have in some cases come to different results. GAULDEN, NIX and DARDEN (1952) and GAULDEN, LOEMKER and DARDEN (1952) used *Chortophaga* neuroblast cells and found that in general P^{32} β-particles from bakelite plaquettes containing the isotope and 125 keV X-rays were equally efficient (mitotic inhibition, number of cells with fragments, and number of single fragments). However, more cells recovered after mitotic inhibition by β-particles than by X-rays, while on the other hand β-particles induced more double fragments than did a similar dose of X-rays. — KIRBY-SMITH

and DANIELS (1953) used chromosome aberrations induced in *Tradescantia* microspores in their investigations, and found an RBE of about 0.7 for P^{32} β-particles in comparison with 200 keV X-rays. This corresponds rather closely to KING's results (1954 a), to be discussed below. However, it should be noted that KIRBY-SMITH *et al.* found that the RBE of Co^{60} γ-rays and P^{32} β-particles were the same, while in *Drosophila* it has been found that Co^{60} β-rays and 175 keV X-rays are equally efficient in the production of recessive sex-linked lethals (MOSSIGE unpublished). Differences in material are therefore of great importance.

The RBE has generally been accepted as constant for any particular effect in any particular organism. However, studies with high energy irradiation of *Drosophila* germ cells seem to indicate that this may not always be true (MOSSIGE 1956, FRITZ-NIGGLI 1958). Since it is known that the RBE may differ considerably when different kinds of lethals are scored [0.5 for recessive and 2.0 for dominant lethals in *Drosophila* after fast neutron treatment (FANO 1944, DEMEREC and FANO 1944)], and since it is not known if the variation in germ cell sensitivity with spermiogenic stage is associated with a shift in type of recessive lethal (e.g. translocation, deficiency, point mutation), a constant RBE for P^{32} β-particles could not be taken for granted in the present experiments.

For these reasons the RBE of P^{32} β-particles has been checked with the present mating procedure, firstly regarding the constancy at different stages of *Drosophila* spermiogenesis (MOSSIGE and OFTEDAL 1958 a), and secondly regarding the absolute value with mature sperm (MOSSIGE and OFTEDAL 1958 b). The first experiment shows that the RBE does not vary significantly during spermiogenesis (Fig. 34). Dosimetry was in this case only relative, by means of dominant lethals. In the second series of experiments, the β-particle doses were measured by means of a special thinwalled ionization chamber. The results show that at the 1000 and 2000 r levels, the RBE is not measureably different from 1.0.

On the basis of this experimental evidence the RBE of P^{32} β-particles has been assumed to be constant and equal to 1.0 for the purposes of the present calculations.

b. *The absorbed fraction of dose (afd)*

The fraction of the radiation dose from the P^{32} contained in the fly which is absorbed in the testes may be determined either theoretically or experimentally. BATEMAN and SINCLAIR (1950) calculated very roughly that about 38 per cent of the total radiation is absorbed in the fly.

KING (1952) made more refined calculations, and states that 9.55 per cent of the emitted radiation dose would be expected to be absorbed at the position of the testes.

The experimental determination of the afd has in all cases been by estimating the dose received by the gonads by means of the genetic effect observed, and comparing this estimated dose with the total dose available, calculated from the physical data and the experimental conditions. These calculations are difficult, and they are not made easier by submitting the fly to radiation from external as well as internal sources (KING 1953, BATEMAN 1955).

The estimate of the effective (KING's "expected") dose from internally deposited P^{32} in ♂♂ allowed to feed for 24 hours on radioactive medium is based on the biological effect observed, and therefore involves a) a value of the RBE, and b) a reference curve for the biological effect in question. KING's estimates have been based on theoretical considerations (1952) [afd=0.0955], on an evaluation of experimental observations on the basis of the dose-effect curve for X-rays published by SPENCER and STERN (1948) [afd=0.02] (KING 1953), and on a reevaluation of these results in conjunction with a new experimental determination of the RBE of P^{32} β-particles, again in relation to SPENCER and STERN's curve [afd=0.094] (KING 1954 a).

Several points in KING's procedure deserve discussion. Firstly, the use of control material from other workers may not be reliable for measuring differences as small as these. Thus, if the data are recalculated for the one point where KING gives the effect of external and of external+internal irradiation (1953, p. 561, 770 rep external), the value of afd is more than doubled. If, on the other hand, the old aggregate dose-effect curve of TIMOFÉEFF-RESSOVSKY and ZIMMER as shown in ZIMMER *et al.* (1937) is used instead of SPENCER and STERN's, the effect of internally deposited P^{32} is not manifest in three of the five observational points. — The flies have been allowed to feed for 24 hours, and have in some cases been mated at the end of the feeding period. No allowance has been made for the shift in distribution of P^{32} during this period. If it is assumed that the abdomen contains on the average $^2/_3$ of the total radioactivity over this period, and the weight of the abdomen is taken to be 40 per cent of the total, the value of the afd will be reduced to one half of the earlier value. — Finally, in some flies the ratio of internal to external dose has been increased by storing the flies before mating them. The period is not specified, but from the radiation data it appears that it may be of the order of three or four days. This

TABLE 10. *Calculation of total dose emitted by P^{32} contained in the abdomen of Drosophila ♂♂, and estimate of absorbed fraction of dose.*

1 eV = $1.60 \cdot 10^{-12}$ erg.
Mean energy of P^{32} β-particle = 0.69 MeV = $1.113 \cdot 10^{-6}$ erg.
1 rep = 93 erg/gram.
Weight of *Drosophila* ♂ = 8.10^{-4} gram.
Weight of abdomen = 40 per cent of total.
Efficiency of counter assembly for live flies

$$0.3475 \cdot \frac{1}{2.40} = 0.145$$

Dose from P^{32} in head and thorax is regarded as genetically not significant. Average P^{32} content in abdomen over first 24 hours after meal, considering excretion and shift in distribution = 42.2 per cent of total immediately after end of meal (Fig. 31).
Dose delivered by P^{32} in ♂♂ by measured activity of (cpm):

$$\frac{(\text{cpm}) \cdot 1.113 \cdot 10^{-6}}{0.145 \cdot 8 \cdot 10^{-4} \cdot 0.4} \text{ erg/gram/min} = (\text{cpm}) \cdot 24 \cdot 10^{-3} \text{ erg/gram/min}$$

or

$$\frac{24 \cdot 0.422 \cdot 60 \cdot 24}{93} \text{ rep/}10^3 \text{ cpm} = 6.55 \text{ rep/}10^3 \text{ cpm}$$

over the first 24 hours after meal, on the basis of initial P^{32} content.
Genetic estimate of absorbed fraction of dose, by means of recessive lethals induced in mature sperm in four different experiments:

	LX	LXIV	LXXXIV	CII
n chromosomes tested	4126	6334	679	1590
Abdominal calculated dose	1690 rep	4975 rep	12350 rep	10640 rep
Genetic estimated dose	180 rep	471 rep	1215 rep	1038 rep
Absorbed fraction of dose	0.10655	0.0947	0.0984	0.0976

Weighted average afd = 9.91 per cent. — This value supersedes that given in MOSSIGE and OFTEDAL 1958 b.

period is long enough to make variation in germ cell sensitivity important.

In experiments in this laboratory (MOSSIGE and OFTEDAL 1958 b, and unpublished) the absorbed fraction of dose has been estimated by means of recessive sex-linked lethals after exposure to internally deposited P^{32} only, in comparison with the standard X-ray value of 2.89 %/1000 r (LEA 1956). Only the P^{32} contained in the abdomen is considered genetically important (KING 1952), and the integral dose over 24 hours has been calculated on this assumption. The calculations of the total dose, and the results from four experiments with recessive lethals induced in mature sperm are presented in Table 10.

As will be seen from the table, the average afd is 9.91 per cent, and

thus very close to the theoretical estimate of KING (1952) of 9.55 per cent. This lends support to the correctness of his calculations [even with the reservations of Dr. ELLIS (quoted by KING 1953) in mind]. The findings are in disagreement with KING (1953) which gives a value of 2 per cent. They also lend no support to the findings of KING (1954 a) of an RBE of P^{32} β-particles of 0.6.

Part of the disagreement noted above surely stems from differences in experimental conditions (stocks used, culture methods, criteria for lethals, etc.) or technicalities (dosimetry, reference curve, etc.), but these factors do not appear to explain all the discrepancies noted. In an attempt to resolve some of these problems, and also the question of the relative importance of the transmutation of P^{32} to S^{32} in the induction of sex-linked lethals, it was decided to perform similar experiments using an isotope of less metabolic importance, but of similar radiation characteristics. For this part of the work, radioactive yttrium was chosen.

B. Radiogenetic effect of the retained Y^{91}

Yttrium-91 was chosen to supplement the P^{32} investigations because it seemed to fill the requirements better than any other radioisotope (see p. 292). Like P^{32}, it is a pure β-emitter, and the maximum energies are of the same magnitude. Yttrium is not metabolically important. It is of fairly low toxicity in rats when administered intraperitoneally (DUBOIS 1956), and about 100 times more killing when administered intravenously (Dr. KYKER in discussion following DUBOIS 1956).

1. Preliminary experiments

Since retention of yttrium after feeding is very low — only 1—5 per cent being retained after 2—4 days, it has been found necessary to inject the radioactive yttrium in the present experiments. The toxic yttrium doses for *Drosophila* ♂♂ have been determined by the injection of stable yttrium chloride, in isotonic solution, with sodium citrate added in equimolar amounts. Injection site has normally been the ventral side of the abdomen. With an injected volume of about 0.06 μl, a concentration of 1.2—0.6 millimole has been found to be tolerated. If *Drosophila* ♂♂ are taken to weigh 0.8 mg, the subtoxic dose is of the order of 8.0 mg yttrium metal per kg body weight, which is the same level of toxicity as that reported for mice and rats (cf. DU BOIS 1956) for intravenous injection. Since in *Drosophila* it is hardly possible to perform anything but an intravenous injection, it is interesting to find that the toxicity levels are so similar in so widely different organisms.

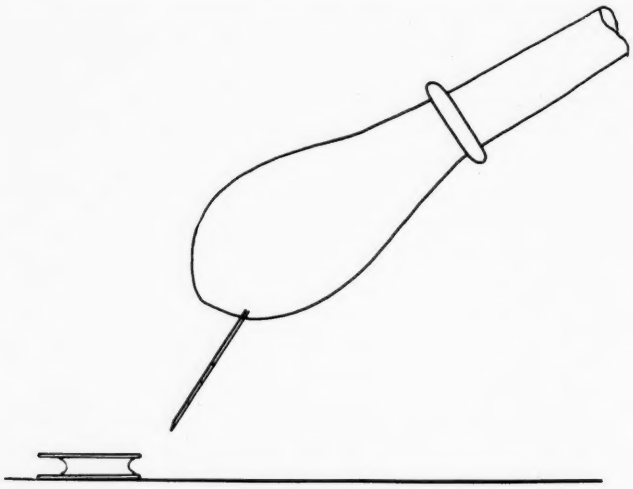

Fig. 28. Injection needle.
The rubber bulb is closed by the handle which consists of a short glass rod. The glass capillary needle is inserted from the outside through a hole made with a thin, round tungsten needle. — The isotope solution is contained in the small glass tube in order to avoid concentration by evaporation during a series of injections.

The injections have been performed with hand drawn, thin walled glass capillary needles. The system used is extremely simple and is illustrated in Fig. 28. The markings on the needle are made with a single hair dipped in finger nail polish, the volumes being determined afterwards by means of radioactive solutions (OFTEDAL 1956 b).

The toxic levels established above will — of course — never be reached by the radioactive yttrium. However, the experiments were necessary in preparation for a genetic control experiment with inactive yttrium. This experiment gave negative results after injection of 0.06 μl 0.6 millimolar solution of yttrium citrate, or about 4 mg/kg. The mutation rate was for Days 1—9 not significantly higher than the control rate commonly accepted. It therefore appears probable that the "interesting changes" in liver cell nuclei after injection of stable yttrium in rats observed by C. LEUCHTENBERGER and reported by LASZLO (discussion following DUBOIS 1956) are not direct changes in the genetic material of the cells, but rather changes reflecting the abnormal physiology of the cells under toxic doses of yttrium.

2. Experiments with radioactive yttrium

The solution of Y^{91} received from Amersham, U.K., for the first series of experiments contained a total solids fraction of 2.1 mg in 3.4 ml. The composition of this fraction is not known exactly, but it depends upon the irradiation time of the target, so some stable Y^{89} is probably present. The remaining part is mainly decomposition products from the resin column used in the separation of the isotopes. Subsequent batches have come from Oak Ridge, Tenn., and have been stated to contain <1 mg/ml of total solids. For unknown reasons — the production method is the same — injections from these batches have not been

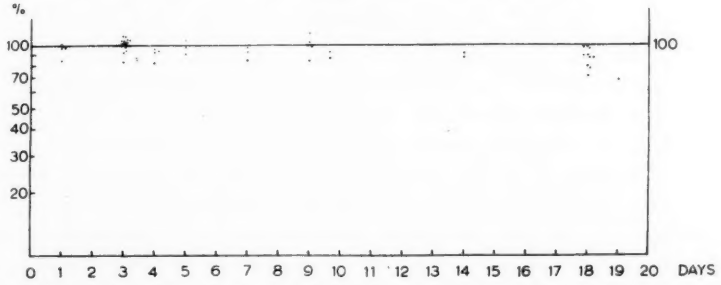

Fig. 29. Retention of Y^{91} citrate after injection. 89 measurements of 37 flies from five different experiments. (LII, LV, LVI, LVIII, CIII.) Semilog scale. Initial Y^{91} content 0.0556 μC—0.0117 μC.

tolerated to the same extent by the flies, and it has often been difficult to obtain a sufficient number of survivors for experiments lasting more than a few days. For this reason, only one long term genetic experiment with Y^{91} has been performed.

The retention of Y^{91} after injection has been measured over different periods. In Fig. 29 the data from several experiments have been combined, and it is seen that there is practically no excretion. Measurements of ♀♀ mated to ♂♂ injected with Y^{91} indicate that no radioactivity is transferred in mating. Injected Y^{91} thus provides a chronic irradiation of only slowly decreasing intensity.

3. Genetic results

The genetic effects have been measured with the same technique as used for P^{32}: ♂♂ containing Y^{91} mated separately to an excess of fresh virgin M-5 ♀♀ each day. The sex-linked recessive lethal mutation curve with time is shown in Fig. 40 (Experiment LVI).

Comparison with the curves obtained after P^{32} feeding shows a gene-

ral similarity in shape, but also some difference in the day to day percentages. Calculations on the basis of the amount of radioactive material contained in the ♂♂ on Day 0, show that while P^{32} causes peak mutation frequencies of 10 per cent per μC on Day 7 and Day 8 in two experiments (XXIII and XLI), the Day 6 mutation frequency for injected Y^{91} is nearly 15 per cent per μC, and Day 10 gives 29 per cent per μC. However, this apparently greater efficiency of Y^{91} becomes uncertain when the difference in retention is considered.

C. The dose-sensitivity product

1. Theoretical

The differences observed between apparent efficiencies of P^{32} and Y^{91} in inducing mutations, the uncertainty involved in comparing the mutation curves obtained by chronic irradiation either from P^{32} or Y^{91} with each other or with that found after acute X-ray or β-particle irradiation, the magnitude of the absorbed fraction of the dose, and finally the question of the relative importance of the transmutation of P^{32} to S^{32} in producing mutations, all emphasize the need for a more general formulation of the dose-sensitivity pattern in chronic irradiation mutation experiments.

On the basis of the experiments presented above, an approach to this many-sided problem has been attempted. In a qualitative way, it has been published elsewhere (OFTEDAL 1959), and the main points will be only briefly recounted here.

The theory rests on three arguments:

a) The brood pattern of mutations found after acute irradiation may be taken as a measure of the sensitivity of the germ cells to radiation at different stages of development.

b) The dose received by the germ cells is proportional to the amount of radioactivity contained in the fly, with corrections for geometrical factors and shifts in distribution.

c) There is a linear relationship between dose and effect.

It must further be assumed that there are no important differences in the spectrum of mutations induced by the different radiations tested.

Finally, it is assumed that the genetic effect of a given dose is the same irrespective of the dose rate at which it is administered (TIMOFÉEFF-RESSOVSKY 1937, p. 73). The recent indications that low dose rate irradiations are significantly less mutagenic in treatments of mouse spermatogonia (RUSSELL, RUSSELL and KELLY 1958) as well as of oocytes

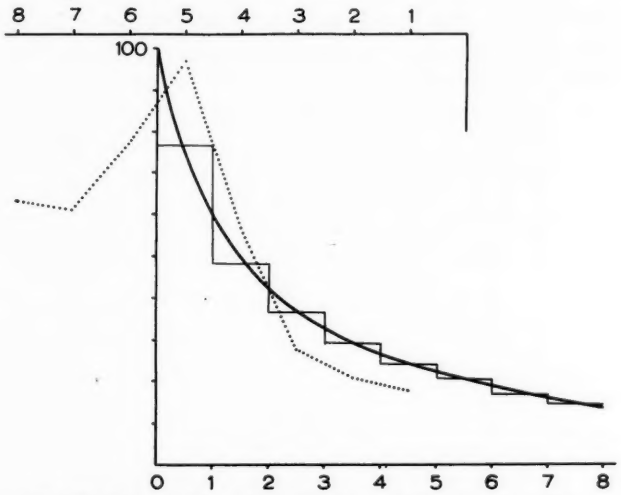

Fig. 30. Data used in calculating the sensitivity product Σ ds.
The heavy smooth curve shows P^{32} retention average for the group of ♂♂ (XXIII). The stepped curve shows the 24 hour values used in the calculations; time scale below, showing days after feeding. The dotted curve shows the sensitivity of the male germ cells, as revealed in the brood pattern after acute irradiation with 1000 r of X-rays; the peak corresponds to $9.67 \cdot 10^{-3}$ per cent per r; time scale above, showing days before maturity.

(CARTER 1958) are not considered relevant since the present calculations do not include spermatogonial stages. Also, the ratio of the dose rates in the present experiments are during the first 24 hours maximally of the order of 500 to one (1000 to one in the second 24 hours) in acute versus chronic irradiation, while in the mouse experiments are used ratios of 10^5—10^6 to one (RUSSELL *et al.*) or 1800 to one (CARTER). Finally, it is not known if the effect is found in *Drosophila* as well as in the mouse.

If these assumptions hold, it is possible to calculate the mutation frequency to be expected after chronic irradiation with inconstant but known intensity.

The dose-sensitivity product

$$\text{afd} \sum_{k=1}^{n} d_n s_{n-k+1}$$

(where afd is absorbed fraction of dose; d is dose in rep; s is sensitivity expressed in per cent sex-linked mutations per r) is the sum of the pro-

TABLE 11. *Acute irradiations.*

Sex-linked recessive lethals in per cent/r. (An expected spontaneous frequency of 0.3 per cent has been subtracted.)

Days	XVI	XL	CI
1	$1.81 \cdot 10^{-3}$	$0.97 \cdot 10^{-3}$	$1.91 \cdot 10^{-3}$
2	2.06	1.33	1.86
3	2.70	2.02	1.92
4	5.66	4.56	4.76
5	9.67	9.45	6.96
6	7.67	10.35	10.30
7	6.20	7.64	11.48
8	6.30	8.28	6.76

XVI 1000 r 175 keV X-rays given to <3 hour old ♂♂ at 1200 hours. (Recalculated from MOSSIGE 1956.)

XL 930 "rep" P^{32} β-particles given to <3 hour old ♂♂ at 2100 hours. Dosimetry by dominant lethals. (Recalculated from MOSSIGE and OFTEDAL 1958 a.)

CI 660 r 175 keV X-rays given to 12 ± 1 hour old ♂♂ at 2130 hours.

ducts of the appropriate radiation dose and the appropriate sensitivity for each day of the period between the beginning of the experiment and the ejaculation of the sperm (k=1 to k=n), and should give the mutation frequency of that day.

The elements of the calculation are presented in Fig. 30 which shows the values used in calculating the sensitivity product for Day 5 on the basis of one P^{32} experiment (XXIII) and one acute X-ray experiment (XVI). The smooth full curve depicts the retention of P^{32} by the ♂♂ in XXIII, and the steps show the average values used for each mating period (Table 12). The dotted curve is the sensitivity after acute irradiation plotted as sensitivity for each day prior to sperm maturity, which is the mirror image of the curve plotted as sensitivity for each day after irradiation (Table 11). The sperm used in the fifth mating period therefore have had a low radiation load on their last day before maturity, but a

TABLE 12. *Average retained isotope fraction in per cent of Day 0.*

No correction for radioactive decay.

Mating period	Experiment		
	P^{32} XXIII	P^{32} XLI	Y^{91} LVI
1	77.5	82.5	98.5
2	48.5	52.5	96.5
3	36.5	34.5	94.5
4	29.5	24.0	92.5
5	24.5	19.0	90.5
6	20.5	15.0	89.0
7	17.0	13.0	87.5
8	14.0	11.0	86.5

Abdominal fraction:

in XXIII: in 1 54 per cent, in 2—8 constant=47.5 per cent.
in XLI: in 1 58.5 per cent, in 2—8 constant=47.5 per cent.
in LVI: in 1—8 constant=80 per cent.

high load four days earlier, when their sensitivity is also high. The relevant product sum becomes (disregarding for the present the question of abdominal retention):

$$d_1s_5+d_2s_4+d_3s_3+d_4s_2+d_5s_1$$

which is to be multiplied with the factor for absorbed fraction of dose. This product may then be compared with the mutation frequency observed on that day in the isotope experiment.

2. Experimental data

The equation of the dose-sensitivity product may be tested on the basis of experimental results. Below follows a discussion of the available material, and estimates based on it. Each factor in the equation will be discussed separately.

a. *Mutations observed*

Several mutation curves after ingestion of P^{32} have been obtained in this laboratory. For the present discussion two of these will be used, since these are the only ones where all other data of interest are available, namely a sufficiently detailed retention curve, and measurements of P^{32} incorporated into sperm. There is one mutation curve available after injection of Y^{91} citrate. The mutation curves for the two P^{32} experiments (XXIII and XLI) are presented in Figs. 25 and 26, and that after Y^{91} treatment in Fig. 40 (Experiment LVI).

b. *Total dose emitted by the isotope*

The dose in the P^{32} chronic irradiations has been calculated on the basis of the average radioactivity content of the group of ♂♂ at the beginning of the experiment. From this the averages have been calculated for each 24 hours over the experimental period according to the retention curve (Table 12, Fig. 30). The averages have subsequently been corrected by taking into consideration the distribution shift from crop to the whole fly occuring during the first hours after feeding (Fig. 31). From the radioactivity content of the abdomen has then been calculated the total dose in rep, according to Table 10, the results being

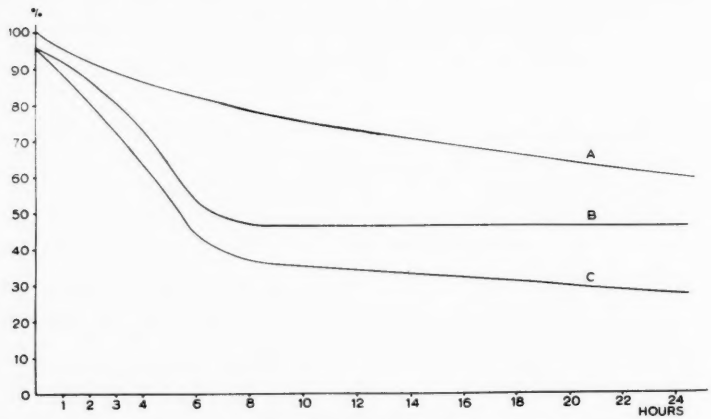

Fig. 31. The shift in P^{32} content of the abdomen of ♂♂ during the first 24 hours after ingestion.

Upper curve: Total retention of P^{32} in live ♂♂. Not corrected for radioactive decay (Fig. 27). Middle curve: Fraction of total contained in the posterior half of the ♂♂ (Fig. 11). Lower curve: The two upper curves combined, to give amount of P^{32} contained in the posterior half.

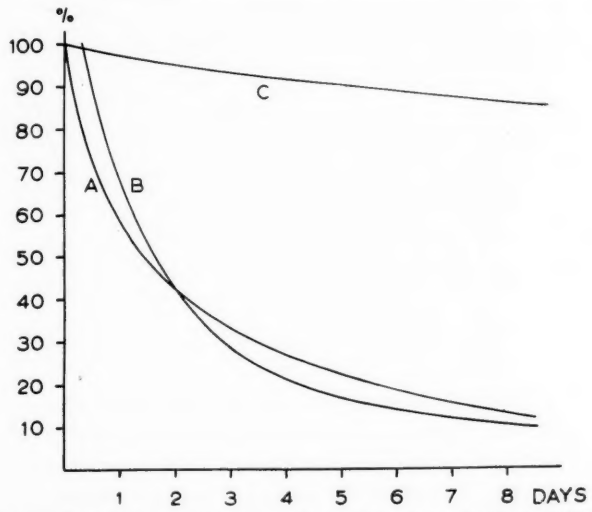

Fig. 32. Isotope biological decay curves used in calculations of the sensitivity product. Curve A: Retention of ingested P^{32} after feeding in the morning (XXIII). Initial P^{32} content 0.131—0.0536 μC. Curve B: Retention of ingested P^{32} after feeding in the evening (XLI). Initial P^{32} content 0.100—0.036 μC. Curve C: Retention of injected Y^{91} (LVI). Initial Y^{91} content 0.039—0.027 μC. No corrections for radioactive decay.

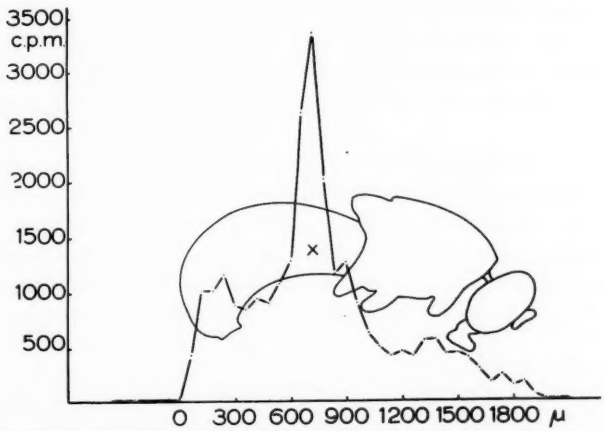

Fig. 33. Distribution of radioactivity in ♂ LV—J, after injection of Y^{91} citrate in the anterior abdomen. × marks the injection site. The area under the curve 0—1000 μ is 77 per cent of the total. Initial Y^{91} content 0.040 μC.

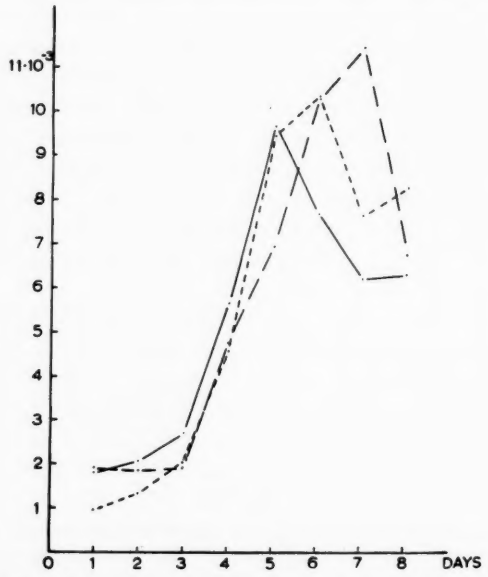

Fig. 34. Sex linked recessive mutations observed after acute irradiations, in per cent per r (or "rep").

Full line: 1000 r of X-rays (XVI), ♂♂ <3 hours old, irradiated in the morning. Short dashes: Calculated dose of 930 "rep" P^{32} β-particles (XL), ♂♂ <3 hours old, irradiated in the evening. Long dashes: 660 r of X-rays (CI), ♂♂ ∼12 hours old, irradiated in the evening.

shown in column 2 of Tables 13 and 14. The biological decay curves for the two P^{32} experiments are shown in Fig. 32, redrawn from OFTEDAL (1959).

The same procedure has been followed for Y^{91}, except that the radioactivity content of the abdomen has been regarded as constant = 80 per cent for the whole experimental period. This factor has been derived from measurements of serial sections of flies on plaquettes (Fig. 33). A more detailed discussion of the distribution of Y^{91} after injection will be published elsewhere. The biological decay curve is shown in Fig. 32.

c. *Germ cell sensitivity*

At present there are three acute irradiation experiments available that can be used for estimating the radiosensitivity of the different stages of the germ cells. The results from these three experiments are presented in Fig. 34 and Table 11. In Exp. XVI (recalculated from MOSSIGE 1956),

TABLE 13.

Days	XXIII		XXIII/XVI		XXIII/XL		XXIII/CI	
	Dose in rep	Obs. mut.	Σ ds	afd	Σ ds	afd	Σ ds	afd
1	4333	0.87	7.84	0.111	4.20	0.207	8.28	0.105
2	2365	1.49	13.21	0.113	8.05	0.185	12.58	0.118
3	1780	0.69	17.79	0.0388	13.63	0.0506	16.12	0.0428
4	1439	2.16	37.18	0.0581	28.31	0.0763	31.23	0.0692
5	1195	2.44	65.22	0.0374	58.40	0.0418	49.80	0.0490
6	999.8	4.59	73.33	0.0626	80.79	0.0568	76.45	0.0600
7	829.1	9.92	77.14	0.129	85.50	0.116	99.07	0.100
8	682.8	3.15	81.94	0.0384	95.20	0.0331	95.24	0.0331

flies were collected in the morning and irradiated at noon. In Exp. XL (recalculated from MOSSIGE and OFTEDAL 1958 b), the flies were collected in the early evening, and irradiated with 930 "rep" from a P^{32} β source at about 22 hours. The dose was determined by biological dosimetry by means of dominant lethals. Finally, in Exp. CI, the flies were collected in the morning and stored without ♀♀ for 12 hours, and were then irradiated with 660 r of 175 keV X-rays.

d. *The absorbed fraction of dose*

The absorbed fraction of dose can be estimated experimentally only by comparing the radiation effect from an internal emitter with the effect of a known dose of externally applied radiation. Therefore, this factor is regarded as unknown and to be estimated in the present calculations.

TABLE 14.

Days	XLI		XLI/XVI		XLI/XL		XLI/CI	
	Dose in rep	Obs. mut.	Σ ds	afd	Σ ds	afd	Σ ds	afd
1	2438	0.10	4.41	0.0227	2.36	0.0424	4.66	0.0215
2	1888	0.76	8.44	0.0900	5.07	0.150	8.14	0.0934
3	1241	1.39	12.72	0.109	8.63	0.166	10.56	0.132
4	863.0	1.18	23.02	0.0513	17.42	0.0677	19.18	0.0615
5	683.2	2.66	40.64	0.0655	36.01	0.0739	31.25	0.0851
6	539.4	4.16	48.60	0.0856	51.90	0.0802	48.12	0.0865
7	467.5	4.65	50.28	0.0925	56.39	0.0825	63.39	0.0734
8	395.5	6.14	51.95	0.118	60.82	0.101	62.86	0.0977

TABLE 15.

Days	LVI		LVI/XVI		LVI/XL		LVI/CI	
	Dose in rep	obs. mut.	Σ ds	afd	Σ ds	afd	Σ ds	afd
1	2890	0.37	5.230	0.0708	2.803	0.132	5.520	0.0672
2	2833	0.70	11.080	0.0632	6.590	0.106	10.783	0.0648
3	2773	0.70	18.655	0.0376	12.295	0.0568	16.113	0.0436
4	2715	2.09	34.620	0.0604	25.220	0.0828	29.505	0.0708
5	2655	2.77	61.845	0.0448	52.010	0.0532	49.068	0.0564
6	2610	5.03	82.623	0.0612	80.870	0.0620	77.860	0.0644
7	2568	4.53	98.993	0.0456	101.303	0.0448	111.923	0.0404
8	2538	6.17	115.220	0.0536	123.180	0.0500	126.820	0.0488
9		10.25	131.25	0.0780	140.0	0.0732	135.0	0.0760
10		8.15	147.5	0.0552	152.5	0.0552	141.25	0.0576
Mean ± 95 per cent interval				0.0570 ± 0.0123		0.0716 ± 0.0281		0.0590 ± 0.0118

3. Results

In Tables 11—15 are presented the numerical values used in the calculations, for observed mutations (m), abdominal dose (d), germ cell sensitivity (s), dose-sensitivity product (Σds), and the derived values of the absorbed fraction of dose (afd). The RBE has been assumed to be 1.0. The absorbed fraction of dose has been estimated on the basis of the observed effect of a chronic exposure, in comparison with the sum of products of dose and sensitivity to acute exposure.

In the equation

$$m_n = afd_n \sum_{k=1}^{n} d_n s_{n-k+1}$$

the different values of *s* are independent of each other, but may all be influenced by common factors, such as culture conditions, total radiation dose used in the acute experiment, and mating program. The different values of *m* will also be independent, with the same kind of limitation, and all be proportional to the same factor, which is the average amount of radioactivity retained in the group of flies, and its variation with time. The dose estimate *d* is based on the retention curve, and since this is constructed from several measurements of the same group of flies, the measurements and therefore the different values of *d* will not be independent. The *afd* values will not be independent, since for each day the *d* and *s* values used on the previous day will again be used. For these reasons it is in general difficult to evaluate the reliability of the results statistically.

The *afd* values for Days 9 and 10 in the Y^{91} experiment (LVI) are based on a graphical extrapolation of the dose-sensitivity product. Since there are no data for the sensitivity on these days, the values cannot be calculated.

In Fig. 35 are presented the values for the absorbed fraction of dose derived from the Y^{91} experiment (LVI). — It will be noted that these ten estimates show fair conformity, which gives an encouraging indication of the soundness of the argumentation as a whole. The 95 per cent expectation limits for each day have been derived from the genetic data from the chronic experiment. This includes only part of the variation, since the other factors in the calculation (genetic data from acute exposure, total isotope content, isotope retention, and isotope distribution) will each have their own error and these have not been

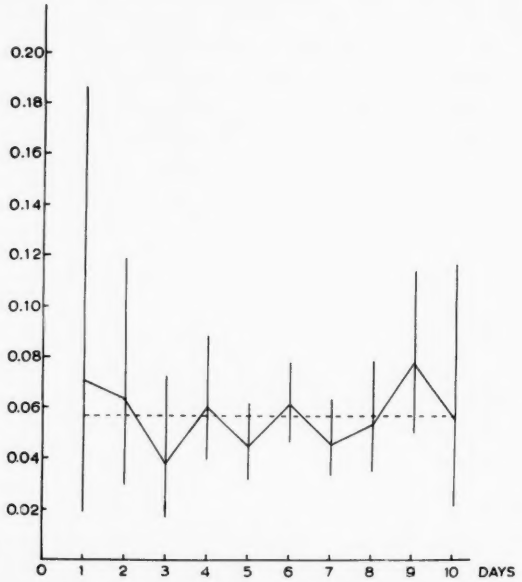

Fig. 35. Absorbed dose fraction calculated on the basis of one Y^{91} experiment over ten days (LVI), and one X-ray experiment with 1000 r dose (XVI).

Vertical lines indicate the 95 per cent expectation interval calculated from the observed mutations after Y^{91} injection. Errors due to variation in the observed values for X-ray mutations, for radioactivity measurements, and for isotope distribution estimate have not been considered.

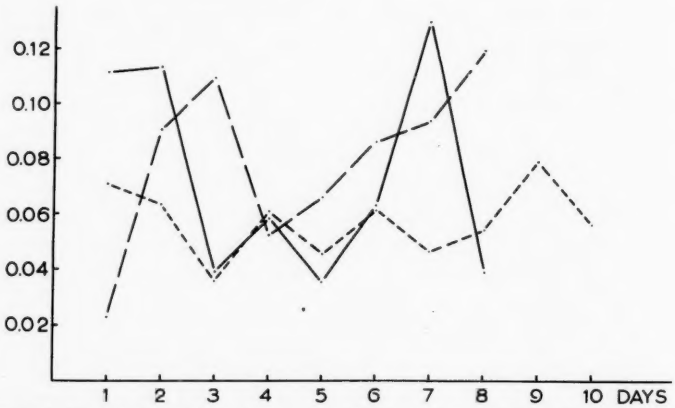

Fig. 36. Absorbed fraction of dose after P^{32} ingestion and after Y^{91} injection. Sensitivity estimated by 1000 r of X-rays (XVI).
Full line: P^{32} Exp. XXIII. Long dashes: P^{32} Exp. XLI. Short dashes: Y^{91} Exp. LVI.

considered. However, the observations may also be regarded as 10 different estimates of the absorbed fraction. If the ten values are treated as independent, and no weighting of the different estimates is attempted, one arrives at an average of 5.70 per cent genetically significant absorbed dose. The 95 per cent expectation limit is ± 1.23 per cent.

In Fig. 36 the values for the absorbed fraction of dose derived from the two experiments with P^{32} (XXIII, XLI) are presented together with those from the Y^{91} experiment discussed above. The values in all three experiments have been calculated on the basis of one common experiment with acute X-rays (XVI). The calculated absorbed dose fractions in the P^{32} experiments will be seen to vary much more than those from the Y^{91} experiment. In XXIII/XVI there are values for the first two days nearly twice as high as the yttrium average of LVI/XVI. Then follow four days where the values are about the same in the two experiments. The other P^{32} experiment (XLI/XVI) shows a low Day 1 value, then high values for Day 2 and Day 3, and then comes down to about yttrium level on Day 4. In the last half of the experimental period there are again high values, in XXIII/XVI an abrupt rise on Day 7, in XLI/XVI a more gradual increase over the whole period Day 4—Day 8.

Additional information on these variations in the estimate may be had, since the absorbed fraction of dose may be calculated on the basis of each of the three acute experiments. These calculations are presented for each of the three chronic experiments in Figs. 37, 38, 39.

In Fig. 37 are shown the three curves calculated from the Y^{91} experiment in combination with each of the acute irradiations. From the figure and Table 15 it will be seen that the averages are almost the same in LVI/XVI and LVI/CI, but that the variability is slightly smaller in the combination LVI/CI. CI is the acute X-ray experiment where 12 hours old ♂♂ were irradiated. In other words, the acute irradiation took place corresponding to mid-mating period in the chronic irradiation experiment. However, the similarity between LVI/XVI and LVI/CI is so great that the curves appear almost identical. In the third combination LVI/XL, there is a definite deviation in the first two — possibly four — days, after which the "normal" level is kept.

In Fig. 38 are presented the data for XXIII/XVI, XXIII/CI and XXIII/XL (Table 13). Again it will be noted that the values for XXIII/CI are closely similar to the ones from XXIII/XVI, although the peak on Day 7 is somewhat less pronounced. The curve based on XXIII/XL, shows the same deviation on the first two days as was noted in LVI/XL. The Day 7 peak is nearly as high in XXIII/XL as it is in XXIII/XVI.

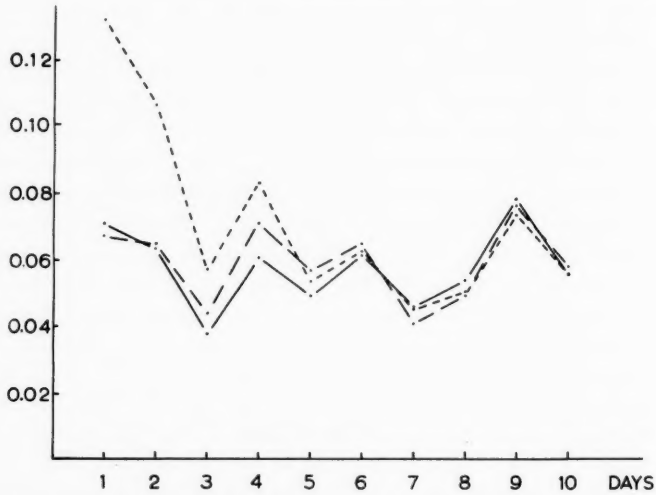

Fig. 37. Estimates of the absorbed dose fraction in one isotope experiment with Y^{91} (LVI) on the basis of three different acute irradiations.

Full line: 1000 r X-rays LVI/XVI. Long dashes: 660 r X-rays LVI/CI. Short dashes: 930 "rep" β-particles LVI/XL.

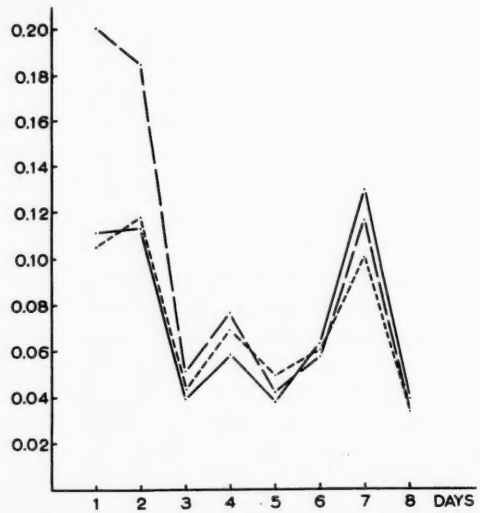

Fig. 38. Estimates of the absorbed dose fraction in one isotope experiment with P^{32} (XXIII) on the basis of three different acute irradiations.

Full line: 1000 r X-rays XXIII/XVI. Long dashes: 660 r X-rays XXIII/CI. Short dashes: 930 "rep" β-particles XXIII/XL.

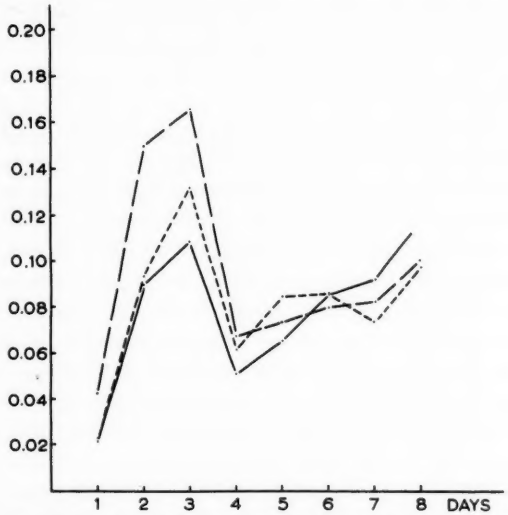

Fig. 39. Estimates of the absorbed dose fraction in one isotope experiment with P^{32} (XLI) on the basis of three different acute irradiations.
Full line: 1000 r X-rays XLI/XVI. Long dashes: 660 r X-rays XLI/CI. Short dashes: 930 "rep" β-particles XLI/XL.

Finally, in Fig. 39 are presented the data for XLI/XVI, XLI/CI and XLI/XL (Table 14). It will be seen that the Day 2—3 peak becomes increasingly predominant in that order, and that the Day 7—8 increase is not prominent in XLI/CI, and hardly discernible in XLI/XL.

4. Discussion

In the equation

$$\text{total dose} \times \text{absorbed fraction} \times \text{mutations/r} = \text{mutations}$$

each factor has been measured independently of the others, and one may therefore regard one or the other factor as unknown, and calculate theoretical values which may then be compared with the values observed or calculated by different methods. The total dose — meaning in this case the efficiency of the counter — is obviously better measured by physical methods rather than by the roundabout way of mutations, and is regarded as known. Attempts to construct an ideal curve for "acute sensitivity" on the basis of dose calculations and the observed induced mutations have proved in vain. The resulting curve shows great oscillations, and very little likeness to those actually observed (Fig. 34).

However, the absorbed fraction of dose, and the sensitivity to chronic irradiation, may each be estimated by means of the other data.

a. *The absorbed fraction of dose*

From the results referred above it is — of course — not altogether easy to ascribe a definitive value to the *afd*. It is not excluded that it may vary with time on account of factors not considered in the present discussion. However, it seems simpler and more economical to accept the more or less constant value obtained from the yttrium experiment (LVI/XVI and LVI/CI) as a basically true estimate of the value.

The similarity of the values, as well as their low standard error, seem to indicate that the method of calculation — as far as it goes — is reliable. If this is accepted, the absorbed fraction of dose in the present experiments is about 5.8 per cent. Deviating values seen in the other experiments may then be discussed against a background of this constant value.

There are, then, three major discrepancies to be discerned in the remaining curves. Firstly, there is the deviation on Days 1—4 in the yttrium experiment results, between LVI/XVI and LVI/CI on the one hand and LVI/XL on the other (Fig. 37). Secondly, there are the high values in the beginning of the P^{32} experiments, on Days 1 and 2 in XXIII and on Days 2 and 3 in XLI (Figs. 38 and 39). And finally, there is the increase on Day 7 in all XXIII curves, and on Days 7—8 in XLI/XVI, possibly in XLI/CI and XLI/XL as well (Figs. 38 and 39).

Before discussing these points further, it should perhaps be emphasized that the genetic effect values for the first 2—3 days are small, and that therefore ratios between them — such as afd — may show relatively large fluctuations of dubious importance. In view of this fact, it is doubtful if the two first-mentioned discrepancies may be given meaningful interpretations.

If — in spite of this — such an attempt should be made, the deviating values in Fig. 37, which are based on the acute external β-irradiation experiment XL, may have their reason in oxygen depletion in the capsule containing the flies during irradiation, causing protection by anoxia (BAKER and SGOURAKIS 1950). If so, it appears that anoxic protection is more effective in the maturer spermatids than in the younger. Experiment XL was performed with the apparatus described in MOSSIGE and OFTEDAL (1958 a) with no provision for aerating the capsule during irradiation. The apparatus has since been modified to meet this requirement.

The high values of afd on the first two days (XXIII) or on the second and third days (XLI) are equally difficult to explain. It may be noted that in the experiments by MOSSIGE and OFTEDAL (1958 b and unpublished) mentioned above, where the afd has been determined after 24 hours exposures of mature sperm, similarly high values have been obtained. It may therefore be that the present analysis of the P^{32} redistribution during the first 24 hours after a meal is not sufficiently refined, and that the dose estimate for this reason is wrong. If, for instance, the dose received by the testes from P^{32} in a dilated crop during the first few hours after a meal is considerably higher than the dose received from the same amount of P^{32} homogeneously distributed in the whole abdomen, the experimental result may be explained.

One more speculative note may be added. Looking at the first 3—4 days only, XL is different from XVI and CI, and XLI from XXIII. XL and XLI have in common that the ♂♂ were collected in the early evening, and irradiated — or fed — at 21—22 hours. The ♂♂ used in the other experiments were collected in the morning. If the diurnal rhythm of eclosion shown to occur in *Drosophila* cultures (BÜNNING 1935, KALMUS 1939) is also reflected in a rhythm of sperm maturation, this may well influence the radiation effect obtained. Experiment CI was designed with these questions in mind.

The abrupt, five-fold increase in germ cell radiosensitivity found by KHISHIN (1955) when comparing the effect of irradiating old larvae with that of irradiating prepupae does indicate that a correlation between germ cell radiosensitivity and other physiological processes may obtain.

The final discrepancy noted in the P^{32} afd curves — the rise towards the end of the experimental period — cannot be explained by any of the above arguments. It is believed that this apparent increase in the genetically significant dose is meaningful, and that its explanation should be sought in the incorporation of P^{32} into DNA, shown to occur in the present experiments on the same days which give these higher mutation rates. In order to examine this explanation more closely, it is necessary to determine the radiation induced fraction of mutations.

b. *Theoretical mutation curves*

If the afd is regarded as known, it is possible to construct theoretical mutation curves based on either one of the sensitivity patterns revealed by the acute irradiations, and the dose calculated from the retention curve. In Figs. 40, 41 and 42 are presented the three curves which may

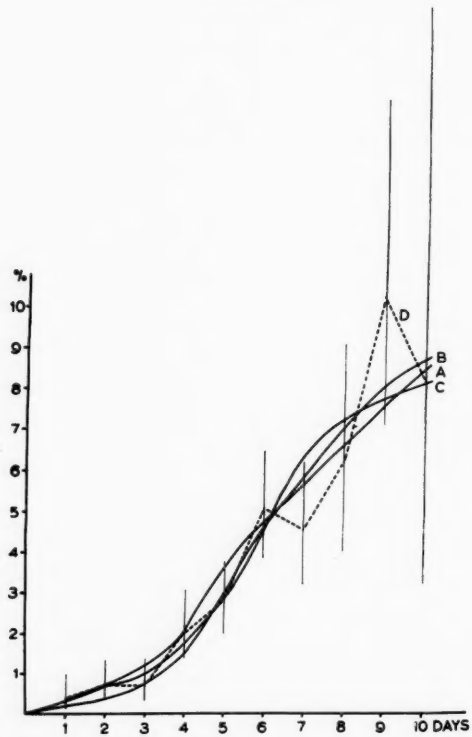

Fig. 40. Sex-linked recessive lethals observed in daily broods after injection of Y^{91} citrate into ♂♂ (Exp. LVI), and theoretical curves based on an absorbed fraction of dose of 5.8 per cent, and on different Σds with s from different acute irradiation experiments.

A: Σds from LVI/XVI. B: Σds from LVI/XL. C: Σds from LVI/CI. D: lethals observed in LVI; vertical lines indicate 95 per cent expectation limit.

be constructed for each of the chronic experiments, together with the observed mutation curve. The afd value of 5.80 per cent is used (mean of LVI/XVI and LVI/CI averages). In this presentation, the conformity between theoretical and observed values in Experiment LVI (Fig. 40) is again seen to be good, and the discrepancy on the first days of LVI/XL as discussed above is seen to be unimportant.

Considering next the two P^{32} experiments, the results as presented in Figs. 41 and 42 show a fairly good fit of the theoretical (curves A, B, C) to the experimental (curve D) values for some portions of the curves.

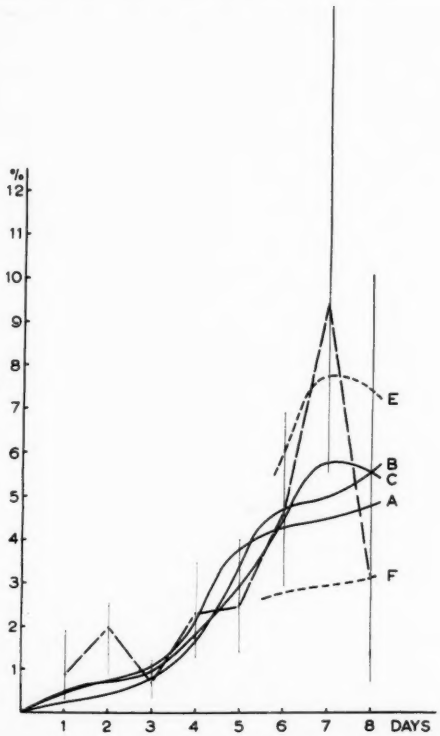

Fig. 41. Sex-linked recessive lethals observed in daily broods after ingestion of P^{32} (XXIII), and theoretical mutation curves based on different combinations of absorbed fraction of dose (afd) and dose sensitivity product (Σds).

A: afd=5.8 per cent; Σds from XXIII/XVI. B: afd=5.8 per cent; Σds from XXIII/XL. C: afd=5.8 per cent; Σds from XXIII/CI. D: lethals observed in XXIII; vertical lines indicate 95 per cent expectation limits. E: afd=7.8 per cent (from LVI/XVI Day 9); Σds from XXIII/CI. This is the highest possible combination for Day 7. F: afd=4.0 per cent (from LVI/CI Day 7); Σds from XXIII/XVI. This is the lowest possible combination for Day 7.

As in the yttrium calculations the curves based on different acute irradiations appear similar, and the differences between them do not appear important. The conformity with the observed mutation curves is again quite satisfactory for the first $^2/_3$ of the observation period. The differences which appeared so striking in the afd presentation on Days 1—3 are not very prominent when the day to day variation in the mutation frequency is considered. It therefore seems probable that the adopted

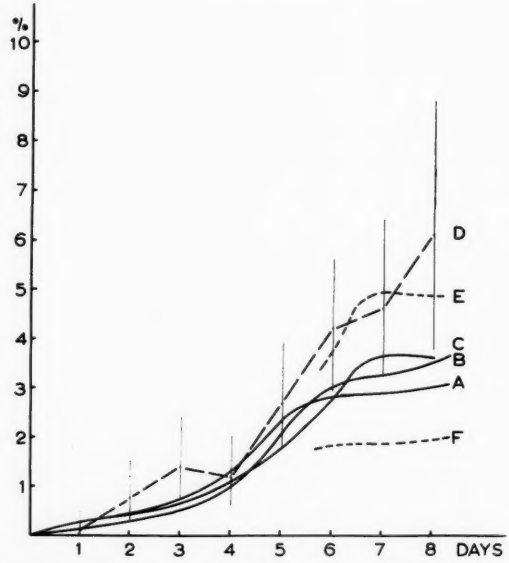

Fig. 42. Sex-linked recessive lethals observed in daily broods after ingestion of P^{32} (XLI), and theoretical mutation curves based on different combinations of absorbed fraction of dose (afd) and dose sensitivity product (Σds).

A: afd=5.8 per cent; Σds from XLI/XVI. B: afd=5.8 per cent; Σds from XLI/XL. C: afd=5.8 per cent; Σds from XLI/CI. D: lethals observed in XLI; vertical lines indicate 95 per cent expectation limits. E: afd=7.8 per cent (from LVI/XVI Day 9); Σds from XLI/CI. This is the highest possible combination for Day 8. F: afd=4.0 per cent (from LVI/CI Day 7); Σds from XLI/XVI. This is the lowest possible combination for Day 8.

way of calculating the expected radiation effect is as satisfactory for P^{32} as it appears to be for Y^{91}.

On Day 7 (Fig. 41) and on Days 6—8 (Fig. 42) the observed mutation rate is higher than expected on the presumption of a constant afd. It is difficult to devise a satisfactory statistical test for the significance of the differences between the observed and the theoretical values. However, the vertical lines in the figures indicate the 95 per cent expectation interval estimated on the basis of the observed mutation data only, calculated according to FISHER and YATES (1953), and the range of the theoretical values is shown by combining the highest values of Σ ds and afd, and the lowest values. The highest Y^{91} afd (excluding LVI/XL) is from the combination LVI/XVI Day 9, and is 7.80 per cent. The highest Σ ds for Days 7 and 8 are found in combinations with CI (XXIII/CI and

XLI/CI). The lowest afd is found in LVI/CI Day 7, and the lowest Σds in XXIII/XVI and XLI/XVI. The values for these extreme combinations have been plotted as curves E and F in Figs. 41 and 42, and it will be seen that although the observed mutation frequencies are still not all exceeded by the high theoretical value, the differences are now materially reduced, and would not unconditionally call for casuative factors other than radiation from the P^{32} contained in the fly.

D. The mutagenic efficiency of the transmutation of P^{32} to S^{32}

1. Calculations

If, however, the differences between the theoretical mutation values and those observed on Days 7 and 8 — though not shown to be statistically significant — are taken to indicate a need for a further causative agent in mutagenesis, the incorporation data discussed earlier provide an obvious possible explanation.

Inspection of Figs. 25 and 26 reveals that for Days 2—6 in both experiments there is an approximately constant number of P^{32} atoms per sperm. This level presumably represents P^{32} which most probably does not occupy DNA sites in the sperm. The grand average for Days 2—6 in both experiments is 22.8 atoms per sperm. If this number is subtracted from the observed value on Days 7 and 8, the true number of DNA P^{32} atoms per sperm is obtained.

Before the amount of P^{32} incorporated into DNA per sperm may be put into a quantitative relationship to the genetic effect to be accounted for, it is necessary to make some assumption about the length of time the average P^{32} atom spends in the genetically significant position. The beginning of this period is — of course — when the P^{32} atom becomes incorporated into the DNA. This may be taken to occur during the first 24 hours after feeding. The end of the period comes not long after fertilization, because as soon as the embryo contains more than one germ cell-to-be, a transmutation induced lethal will result in a gonadal mosaic, which will not be picked up by the genetic system used. Since under the mating procedure used the ♀♀ will have laid the bulk of their fertilized eggs in two or three days (relatively few flies eclode in these bottles after the twelfth day), on the average, 24 hours may be assumed to pass between the end of the mating period and fertilization. If, therefore, time lag in incorporation and fertilization are assumed to compensate each other, we are more probably underestimating than overestimating the period available for genetically effective transmutations.

The mutations observed in the present experiments are recessive sex-linked lethals. Induced recessive lethals are distributed on the different chromosomes roughly in proportion to the lengths of their euchromatic regions (TIMOFÉEFF-RESSOVSKY 1937, KÄFER 1952 for radiation induction; AUERBACH and MOSER 1953 for chemical induction). Since the sex chromosome represents about 1/5 of the total euchromatic material in *Drosophila melanogaster*, the total frequency of recessive lethal mutations must be presumed to be about five times as high as that observed.

In order to estimate the mutagenic efficiency of the transmutation process of P^{32}, it is therefore necessary to compare the calculated number of transmutations over the period in question, with the total frequency of recessive lethal mutations not accounted for by radiation, or five times the frequency observed in the X-chromosome. The same result would be obtained by comparing the sex-linked lethal frequency with the number of P^{32} atoms transmutating after incorporation into the X-chromosome, which may be taken to be 1/5 of the total.

The method of calculation is illustrated in Table 16, using XXIII Day 7 as an example. The frequency of radiation induced mutations to be expected has been taken from curve A in Fig. 40. In the rest of the table are given the mutagenic efficiencies for Day 7 (XXIII) and Days 7 and 8 (XLI). The expected radiation induced mutations are calculated on the basis of different sensitivity products and an afd of 5.80 per cent, and on the combination of the highest afd and Σ ds, and the lowest, as illustrated in Figs. 41 and 42. For Day 8 (XXIII) observed mutations are lower than expected, and calculation is impossible. For Day 6 (XLI) observed mutations are unexpectedly high, but incorporation is hardly above the control value, so no efficiency estimate can be given.

The table shows that the efficiency of the P^{32} transmutation in the DNA of *Drosophila* sperm for inducing recessive lethals is between 1/67 and 1/157, when calculated on the basis of sex-linked lethals in the present material. The unweighted average of the observed values is one recessive lethal per 103.7 transmutations. If the worst possible combinations of afd and Σ ds are used, extreme values of 1/42 and 1/298 are obtained.

In these calculations the possible role of heterochromatin has not been considered. It may well be that the heterochromatin has the same incorporation of P^{32} as euchromatin, but that the genetic effect of transmutation in these regions is less drastic (HANNAH 1951, SCHULTZ 1956). If the incorporation is taken to be the same, and the genetic effects of transmutation to be absent, the over all effect on the present calcula-

TABLE 16. *Calculation of mutagenic efficiency of transmutation.*

XXIII Day 7 — n atoms per sperm 134.4
— "background" 22.8
DNA P^{32} per sperm 111.6 atoms

n transmutations in 7 days = $111.6 \times 0.292 = 32.5$
Observed mutations 9.92 % sex-linked recessive lethals (XXIII)
radiation induced 4.45 % „ „ „ (XXIII/XVI)
not accounted for 5.47 % „ „ „
which gives a total of $5.47 \times 5 = 27.35$ per cent lethals

Mutagenic efficiency: $\frac{0.2735}{32.5} = \frac{1}{119}$

Mutagenic efficiency

	low	XVI	XL	CI	high
XXIII Day 7	$\frac{1}{93}$	$\frac{1}{119}$	$\frac{1}{131}$	$\frac{1}{157}$	$\frac{1}{298}$
XLI Day 7	$\frac{1}{42}$	$\frac{1}{67}$	$\frac{1}{86}$	$\frac{1}{120}$	—
XLI Day 8	$\frac{1}{67}$	$\frac{1}{89}$	$\frac{1}{107}$	$\frac{1}{111}$	$\frac{1}{224}$

Average for middle 9 values $\frac{1}{103.7}$

tions will be to increase the estimate of the efficiency in the euchromatic region by some 20—30 per cent. In view of the uncertainties already involved in the calculations, as well as in the above assumptions regarding heterochromatin, no attempt to include these latter corrections has been made.

It is also assumed in the above arguments that all the dominant lethals are radiation induced, and none caused by transmutation. This may not be true. However, it is suspected that transmutation will be less effective than ionizing radiation in the production of dominant lethals, since single hit events only are to be expected and that the correction therefore will be unimportant.

2. Discussion and conclusions

Earlier studies — after the completely negative results of LAW (1939) in *Drosophila* — by POWERS (1948) on *Paramecium*, by GILES and BOLOMEY (1948) on *Tradescantia* anthers, and RUBIN (1950) on *E. coli* indicated that P^{32} treatment can be more effective than is to be expected

from the ionization by the emitted β-particles. Subsequently, definitive data on the biological effects of the transmutation of radioisotopes have been obtained with microorganisms. Thus, it was first shown by HERSHEY *et al.* (1950) for bacteriophage, and by FUERST and STENT (1956) for bacteria, that P^{32} transmutation is effective, and by HUNGATE *et al.* (1952) for *Neurospora* conidia that S^{35} gives the same effect. STRAUSS *et al.* (1956) have compared the efficiencies of P^{32} and S^{35} when incorporated into *Neurospora* conidia.

The experimental procedures used in these studies are based on either one of the following two principles. In the first type of experimental design — used by HERSHEY *et al.* (1950), STENT (1953), and others — the organisms to be studied are allowed to incorporate the radioisotope during a period of growth. After washing and dilution in order to reduce the radiation load from un-incorporated isotope in the medium, the organisms are stored for different periods of time, under conditions of non-metabolism, after which growth is again induced and radiation effects scored. The results are then compared with the appropriate controls, and with the physical decay of the isotope in question, and the transmutation efficiency evaluated.

In the second type of procedure — used by HUNGATE *et al.* (1952) — the irradiation of the organisms is kept constant by the use of a constant concentration of radioisotope in the medium. The differential treatment is obtained by varying the specific activity, whereby differences in the incorporation of the radioisotope are obtained. Intergroup comparisons after identical exposure periods then provide basis for calculations of the mutagenic efficiency of the radioisotope disintegration.

In this way efficiencies of about 0.1 for the disintegration of P^{32} have been obtained both for the "suicide" of bacteriophage (HERSHEY *et al.* 1950, STENT 1953, STENT and FUERST 1955) and for the inactivation of bacteria (FUERST and STENT 1956). Indeed, this figure of 1/10 is used by LEVINTHAL (1956) for the deduction of the number of P^{32} atoms incorporated per bacteriophage particle from the number of inactivations observed.

The value of 1/10 is found for room temperature. If the physical decay takes place at —196° C, the efficiency is reduced to about 1/20, while if it is raised to 65° C, the efficiency goes up to about 1/3 (CASTAGNOLI and GRAZIOSI 1954, 1957, FUERST *et al.* 1956). The principle of inactivation by radioisotope disintegration has been used to study the mode of replication (STENT 1955) and the organization (LEVINTHAL 1956) of the genetic material of bacteriophages.

This list of successful investigations in microorganisms has no parallel in plants or Metazoa. BATEMAN's (1955) somewhat pessimistic views have been quoted in the introduction, and in a recent review by STRAUSS (1958) the results of KING and WILSON (1954) are the only data from work with higher animals referred to. These authors compared the effects on the fecundity and fertility of *Drosophila* when the flies were raised in a gamma field at distances from the source so determined that the dose should be the same as that absorbed in the gonads of similar groups of flies raised on medium containing P^{32}. In comparison with the appropriate controls, the flies on P^{32} showed greater effect, and this was ascribed to the recoil of the atoms of P^{32} disintegrating within the body of the flies. The flies were raised on a synthetic medium, and partly without temperature control, and the experimental difficulties appear to have been considerable. The numbers are small, and no quantitative estimate of the effects is attempted by KING and WILSON. The case is considered to be doubtful by STRAUSS.

It is suspected that the limited success of the attempts of BATEMAN and of KING and WILSON regarding quantitative results stems mainly from the mode of administration of the isotope. The culture of *Drosophila* larvae on radioactive medium leads to the exposure of the gonads of the developing fly to relatively high doses of external radiation, as well as to that coming from the ingested isotope. The ratio of transmutation induced mutations will therefore be lower than what might be expected in experiments designed to reduce external irradiation. The situation is similar to the second of the experimental procedures discussed above, but is complicated by the difficulty of calculating the total dose to which the gonads of the fly are exposed, and of duplication by means of external radiation sources.

In the system used in the present investigation, the experimental design approaches the first of the two procedures discussed above. The fly is exposed to radiation from the food for an hour or less, after which exposure only from the ingested isotope obtains. A period of comparatively rapid excretion follows after the meal, and causes a corresponding decrease of the radiation from the internally deposited isotope. However, the latter aspect also reduces the specific activity of the isotope available for incorporation into the genetic material, and therefore may cause a less favorable ratio between the mutations resulting from the two processes. The ratio could possibly be changed in the desired direction by keeping the flies on a low phosphorus diet for a relatively short period after the meal — in order to obtain maximum incorporation,

and on a high phosphorus diet for a subsequent longer period — in order to remove un-incorporated P^{32} and thereby reduce radiation background. The original plans for the present experiments included measures to this effect, but they were dropped in order to avoid the possibility of disturbing side effects. The theoretical mutation curves of Experiments XXIII and XLI (Figs. 41 and 42) also seem to indicate that something might have been gained, since the ratio of transmutation induced to radiation induced mutations is about 0.89 to 1 in XXIII Day 7, which has the higher retention, and 0.75 to 1 in XLI Day 8, which has the lower retention (Fig. 32).

The present study has amongst its results an estimate of the mutagenic efficiency of the transmutation of P^{32} to S^{32} in *Drosophila* DNA of 1/100. This value as calculated in the present study has an unestimated and possibly large error, which stems rather from the unknown factors in the calculations of the number of P^{32} atoms per sperm (sperm consumption per offspring, efficiency of the DNA-P^{32} isolation procedure) than from the evaluation of genetic and radiation factors (distribution of lethals, RBE, radioisotope distribution). These sets of factors are common to all the calculations, and the conformity of the efficiency values as presented in Table 16 may therefore be misleading.

If, however, the mutagenic efficiency of 1/100 is accepted for DNA-P^{32} transmutation and sex-linked recessive lethals in *Drosophila*, this value differs by an order of magnitude from that established for the "suicide" effect in bacteriophage (STENT and FUERST 1955) and for inactivation in bacteria (FUERST and STENT 1956). This difference may be caused by a higher organization of the genetic material in *Drosophila*, but also by dissimilarities between the effects scored in flies and in microorganisms. The present material does not provide basis for a choice between these two alternatives.

Note added in proof: The dose rate of the Co^{60} source used in the RBE experiments (p. 294) has recently been remeasured by means of intracavity ionization chambers, and has been found to be higher than originally calculated on the basis of chemical dosimetry. According to the new dose measurements, the RBE will be considerably under 1.0.

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SUMMARY

(1) The retention of P^{32} by *Drosophila* ♂♂ after a single meal of contaminated sugar solution is shown to be adequately described as a sum of three exponential processes.

(2) All three processes are influenced by diet and/or mating.

(3) Differences in retention between groups on diets of low and normal phosphorus content are in the expected direction. On a low phosphorus diet there is seen a) a higher retention of P^{32}; b) a higher tagging of sperm and seminal fluid, which is reflected in more sustained mating loss; c) a higher correlation between mating transfer of P^{32} and offspring production.

(4) A constant distribution of P^{32} between fore and aft halves of the ♂ is reached about seven hours after a single meal.

(5) P^{32} incorporated into the DNA of mature sperm is recovered seven or eight days after a single meal, under conditions of excessive mating, which coincides with maximum genetic effect. The number of P^{32} atoms per sperm is calculated.

(6) Radioactive yttrium is retained completely after injection in the form of citrate.

(7) On the basis of the known germ cell pattern of sensitivity to acute irradiation, and the total dose calculated to be emitted by the retained Y^{91}, the fraction of dose absorbed in the testes of the ♂ is calculated to be 5.8 per cent.

(8) On the basis of this absorbed fraction of dose, the pattern of sensitivity to acute irradiation, the retention curve for P^{32}, and the shift in the distribution of P^{32} the mutations to be expected from P^{32} radiation are calculated.

(9) Discrepancies are shown to occur between the observed genetic effect and that calculated from the above data. These discrepancies are found on days with high incorporation of P^{32} into sperm DNA, and transmutation of P^{32} to S^{32} is taken to be responsible for the unexplained genetic effect.

(10) The efficiency of the transmutation in producing recessive lethals is found to be one in one hundred.

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ON THE INHERITANCE OF ACIDITY IN CULTIVATED APPLES

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I. INTRODUCTION

WHEREAS the improvement of short-termed plants like wheat, maize and others is greatly favoured by a fairly rapidly increasing knowledge of the genetical constitution of these plants and the mode of inheritance of the agronomically important characters, the breeding work with fruit trees is still considerably hampered by our limited experience and incomplete information.

It is only a slight exaggeration to say that we are still left to work in a pre-Mendelian fashion by crossing the best with the best in a rather haphazard way just as has been done for hundreds of years.

The long cycle of generation, amounting to 6—12 years in apples as well as the space the trees require are of course the main reasons why we have not advanced further. But this is probably not the whole explanation. Obviously, most properties that have been hitherto studied have a very complicated genetical background, especially as regards apples. Extensive and painstaking investigations by *e.g.*, WELLINGTON, 1924; CRANE and LAWRENCE, 1933, and SCHMIDT, 1947, show that there is usually a more or less pronounced correlation between parents and offspring in such characters as earliness of ripening, hardiness, fruit size and so on. But the range of variation is always very broad and mostly continuous, showing that these properties are polygenically determined. Thus, the exact genotypical constitution of the parents has remained obscure, and the results of further crosses are, therefore, always difficult to anticipate.

Only in some very few cases has it been possible to attribute the variation found among the cultivated apples to distinct major factors or oligogenes. After self-fertilization of the variety *Rev. W. Wilks*, CRANE and LAWRENCE (1933) found albinotic plants in a frequency that pointed towards monofactorial inheritance. The same was true for the

inheritance of the high anthocyanin content of the wild species *Malus Niedzwetskyana* according to LEWIS and CRANE, 1938.

In other crosses, however, characters similar to these have shown a more complicated picture and one that is not so easily interpreted. There has been a tendency to throw the blame for this meagre success of the genetical analysis onto the chromosomal set-up of the subfamily *Pomoideae*, its basic chromosome number 17 being supposed to be derived from types with a lower number. The main difficulties, however, seem to be the extremely rich variation found among cultivated apples, probably going back to their supposedly poly-phyletic origin, together with their high degree of heterozygosity maintained by a rather obligate cross-fertilizing mode of reproduction.

Thus, we find ourselves at the beginning of a purposeful plant breeding based on genetical knowledge. We have, therefore, still to welcome all contributions to this knowledge, and that is why the analysis presented in this paper may be of some interest, although it may appear very incomplete and old-fashioned to geneticists working with more favourable organisms.

During the summer of 1957, the author initiated some studies on correlation phenomena with the idea of elaborating more efficient methods of selection. A correlation between the acid content of leaves and fruits in apples, for instance, might render it possible to select types with a desired acid level from small seedlings long before they start fruiting.

In some cross populations there was then found a sharp discontinuity with regard to the pH of the fruits, which gave an ideal fit with a 3 : 1 segregation. In the population *Wealthy*×*Cox's Orange* there were found 14 seedling trees with a fruit pH between 3.0—3.4 and 4 with a pH between 3.9—4.1. In the population *Jonathan*×*Cox's Pomona* there was a similar distribution with 25 trees having a fruit pH between 3.0—3.3, and 9 between 4.0—4.4. On the other hand, the population *Cox's Pomona*×*Boiken* did not segregate any sweet apples with a high pH. From some collected fruits it was found that those with a pH around 4 were typical "sweets" with a flat, insipid taste more or less lacking in acid flavour, whereas those with a pH slightly over 3 were of the more normal acid type. Last summer these studies were continued and led to a confirmation of the preliminary findings as presented in the following sections of this contribution.

II. MATERIAL AND METHODS

The seedling trees from which the fruits were taken in this study belonged to the breeding material of the station. In all, 21 different cross combinations were investigated, usually as samples of 25 trees from each population. The crosses giving rise to these populations had been made during the years 1942—1948, most of them in 1946. The average age of the trees was, therefore, 10—12 years. Most of them had been fruiting for one or more seasons already.

The numbering of the seedlings used later on in the paper is based on the following system. The first two figures denote the number of the row, and the last two figures the number of the tree in the row.

The juice was pressed directly from the apples with the aid of a kitchen fruit press. This was very easy, even as regards the firm, unripe apples, if they had been frozen at — 25° C for a day. They could then be collected and stored at the low temperature until time was available for the analysis. Most of the analyses were made directly on this juice. For the determination of ascorbic acid fresh apples had to be used, as this substance is more or less completely oxidized when the apples thaw. The pH was determined with a glass electrode. The total acidity was titrated with 0.15-n NaOH against phenolphthalein as indicator.

When large numbers of seedlings were to be assayed with regard to acidity it was found difficult to taste fruits from all trees, especially before the fruit was ripe. As a routine method for classifying the seedlings into acid or sweet, a drop of a solution of bromo-cresol green was put onto a fresh section of the fruit. If the indicator remained green the apple was sweet, if it turned yellow the apple was acid. This "tooth-saving" method was found to be both accurate and efficient.

For the more detailed analysis presented here, for instance in Table 4, the following methods were used: *Firmness of the fruit:* Magness' fruit pressure tester (HALLER, 1941). *Vitamin C:* Extraction with 5 % metaphosphoric acid in a Turmix, titration with dichlorophenolindophenol. *Tannin:* The arbitrary method used at Long Ashton Research Station determining the total amount of permanganate-reducing compounds. The juice is titrated with $KMnO_4$, using indigo carmine as indicator. *Sugars and Acids:* Paper chromatography, to be described in more detail below. *Titrable acidity:* The more exact method worked out at Long Ashton Research Station (*cf.* BURROUGHS, 1946), using dilute juice and bromo-thymol blue and phenol red as indicators. *Dry matter content:*

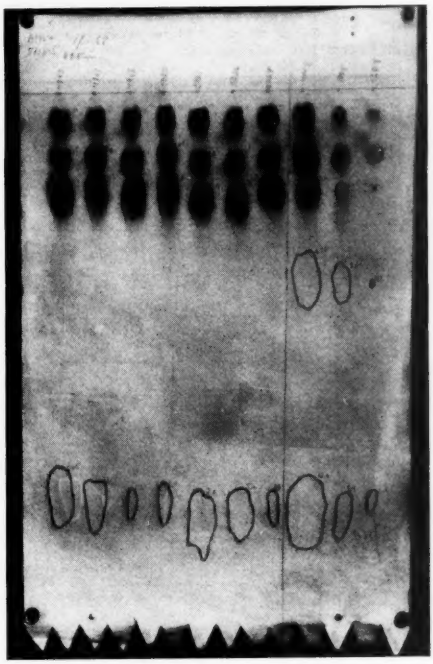

Fig. 1. Chromatography paper prepared as described in the text. The first seven samples consist of apple juice, nos. 3, 4 and 7 being of the sweet type, the other acid. The last three samples are standard mixtures of sucrose, glucose, fructose, quinic acid (not found in the nearly ripe apples) and malic acid.

Content of solids, determined refractometrically. *Viscosity:* By means of an Oswald viscosimeter with a value for pure water of 20 seconds at 20° C. *Colour:* Determined with a spectrophotometer as extinction at 460 mμ, using a 1-cm. cuvette. *Turbidity:* Relative turbidity determined by means of a simple turbidimeter described in another connexion (NYBOM, 1959).

The separation of sugars and acids on filter paper involved the following procedure. As paper, Whatman No. 1 was used. The following solvent mixture was found to give the best separation of the individual sugars (*cf.* BAYER, 1958): n-Butanol 57 ml : Formic acid 11 ml : Water 5 ml. This mixture does not separate into two phases and can, therefore, be used immediately after mixing.

After 19 hours' descending development, the papers were dried and sprayed with bromophenol blue solution (RANSON, 1955), and the acid spots were drawn with a pencil. The papers were then sprayed with a Benzedine reagent (LINSKENS, 1955, p. 66) and the sugar spots made

TABLE 1. *The distribution of apple seedlings according to fruit pH.*

Cross combinations	pH of the fruit juice												Segregation	
	2.8–3.0	3.0–3.2	3.2–3.4	3.4–3.6	3.6–3.8	3.8–4.0	4.0–4.2	4.2–4.4	4.4–4.6	4.6–4.8	4.8–5.0	5.0–6.0	Acid	Sweet
Wealthy×Cox's Orange (1957)	1	9	3	1		1	1	2					14	4
Jonathan×Cox's Pomona (1957)	1	8	16				4	4	1				25	9
Cox's Pomona×Jonathan (1958)		5	13	2			2		3				20	5
Cox's Pomona×Filippa		1	11	5	2		1		2		1	1	19	5
Filippa×Oberländer Himbeerapfel		13	9	2			1	1					24	2
Filippa×Oranie		13	7					4					20	4
Filippa×Dronning Louise	3	14	5					3					22	3
Cox's Pomona×Dronning Louise	1	9	8	1				4		1			19	5
Cox's Orange×Ontario	5	6	4	1	2				2				18	2
Egremont Russet×Ontario	4	4	3	1	2		2	2		2		1	14	7
Yellow Transparente×Early McIntosh	6	14	1		1		1	1		1			22	3
Yellow Transparente×Roter Ananasapfel		23	17	3			2	1	3	1			43	7
Yellow Transparente×Worcester Pearmain	4	14	4				3						22	3
James Grieve×Worcester Pearmain		7	13		1			1	1	1			21	3
Laxton's Superb×Oranie		2	10	5	3	2		1		1		1	20	5
Roter Ananasapfel×Gyllenkrok's Astrachan	1	10	3	1	1	1	1	2					16	4
v. Zuccalmaglio's Reinette×McIntosh	1	13	5	1			1	1	2	1			20	5
Sum of all segregating populations	27	165	132	23	12	4	19	27	14	8	1	3	359	76
Cox's Pomona×Boiken (1957)	2	8	1										11	
Boiken×Cox's Pomona (1958)		16	8										24	
Boiken×Pigeon Rouge	2	10	5										17	
Boiken×Cox's Orange	1	9	9	1									20	
Signe Tillisch×Dronning Louise	1	11	12	11	6	3							44	
Åkerö×Lord Lambourne		19	6										25	
Sum of all non-segregating populations	6	73	41	12	6	3							141	

visible by 15 minutes' treatment at 100° C. The spots were then measured with a planimeter and compared with standards. In this way sucrose, glucose, fructose and malic acid — in unripe fruit also quinic and chlorogenic acid — could be analysed on the same paper (*cf.* Fig. 1).

III. RESULTS

During the summer of 1958 samples from some 20 new populations were investigated with regard to the pH of the fruit juice. The aim was

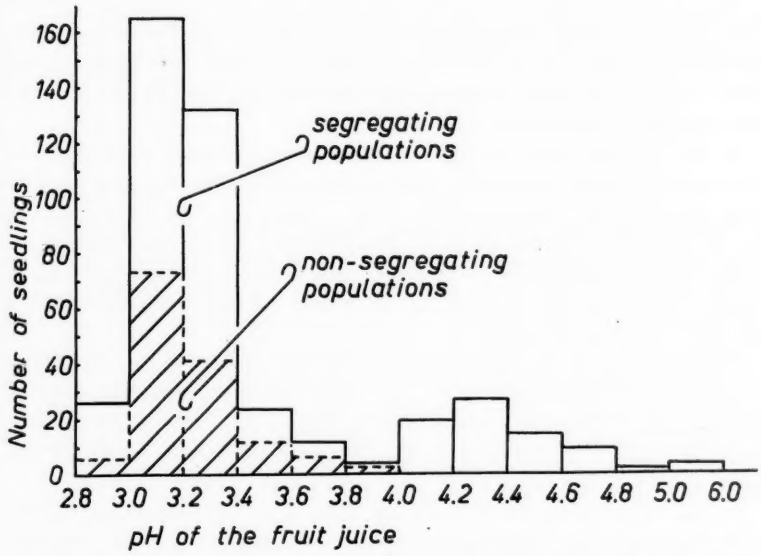

Fig. 2. Diagram showing the total pH distribution of apple seedlings in various cross combinations. Cf. text.

to find out whether the sharp distribution into acid and sweet types was also to be found in other cross combinations and, if so, whether the same simple interpretation, *i.e.* a monofactorial segregation, could be generally applied. In most cases a sample of about 25 trees, chosen at random, were analysed from each population. The results are presented in Table 1. As seen, most of the populations did segregate into the same groups and in about the same ratios as before. The values

TABLE 2. *The segregation into acid and sweet apples in four cross combinations (cf. text).*

Population:	Distribution		P-value
	Acid	Sweet	
Filippa×Oberländer Himbeerapfel	89 (82)	20 (27)	$0.3 > p > 0.2$
Jonathan×Cox's Pomona	103 (105)	37 (35)	$0.8 > p > 0.7$
Laxton's Superb×Oranie	49 (45)	11 (15)	$0.5 > p > 0.3$
Yellow Transparente×Roter Ananasapfel	61 (58)	16 (19)	$0.7 > p > 0.5$

from 1957 are also included in the table. Some new populations, in which *Boiken* was one of the parents, only contained acid types. In the same way the Danish variety *Signe Tillisch* and the Swedish *Åkerö* only gave acid offsprings.

As the samples were rather small the segregation in each has not been treated statistically. The total sums of all the segregating populations are presented graphically in Fig. 2. There are two clear peaks in the distribution, and the most obvious interpretation is that there is an ordinary 3 : 1-segregation. The sweets would then represent the homozygous recessives and the acid group would contain the heterozygotes and the homozygous acids.

Altogether there are 359 acid seedlings to 76 sweet, *i.e.* there is a shortage of sweet seedlings. The ratio acid : sweet corresponds to 4.7 : 1 instead of 3 : 1. Now, it is not so rare to meet with such a deficiency of recessive individuals. It should also be noted that these seedlings had been subjected to various kinds of selection during their lifetime.

As the segregation ratio, however, seemed rather important for the interpretation of the distribution, some of the populations were studied more extensively. As the first samples were too small to permit any test of the homogeneity of the material, the first step was to control whether, for instance, the highly deviating ratio 24 : 2 after *Filippa×Oberländer Himbeerapfel* was typical of that population.

All trees of four populations were classified into acid and sweet with the aid of the bromo-cresol green method described above. All the sweets were controlled by taste. The difference in taste is so pronounced that there is rarely any hesitation as to whether a given apple belongs to the acid or to the sweet group. A condition for this is that the analysis is carried out before the apples ripen, *i.e.* during July or early August. The results of these counts are shown in Table 2. The values

expected according to a 3 : 1-distribution are given within brackets. The p-values for agreement with such a distribution are given in the last column.

These additional data are in very good agreement with the hypothesis that the variation in acidity observed is caused by a single Mendelian factor. There is, however, especially in Table 1, a marked deficiency of recessives for which no explanation can be given for the moment. The difference in the average segregation ratio between the two Tables is not significant (χ^2 for identity=2.40; $0.2 > p > 0.1$).

It may be added here that this general interpretation is also supported by less systematic observations on other cross combinations. The homozygous nature of *Åkerö*, for instance, was confirmed in the combination with *McIntosh*. In this population there were 61 acid and no sweet seedlings fruiting this year.

The chemical analysis

A variation in pH or titrable acidity of the fruit juice might be brought about either by changes in the content of organic acids, in the first place malic acid as far as apples are concerned, or by changes in the concentration of neutralizing mineral components, primarily potassium. In order to investigate further the nature of the variation in acidity just described, juice samples from the segregating progenies, as also from a number of known varieties, were analysed by means of paper chromatography. As might be expected, it was found that the types with a high pH invariably contained much less malic acid than did the more acid ones. The results of two series of analyses are shown in Table 3.

These analyses were made on the 3rd of August, 1958. Therefore, the juice samples still contained a fair amount of quinic acid as seen in the table. This acid disappears from the juice of dessert apples later on, and in the fully ripe apple it is usually not detectable with these methods. It is of interest to record that the genetic factor responsible for the reduction in the malic acid content does not influence the formation of quinic acid.

The same is true for another related acid, chlorogenic acid, which was only present in traces in the juice. On the filter paper the quinic acid spots are found between the sugars and the malic acid (*cf.* Fig. 1). The chlorogenic acid travels somewhat faster than the malic acid, how-

TABLE 3. *pH and acid content of samples from two cross populations.*

Filippa × *Oberländer:*	pH	‰ in the fruit juice: Malic acid	Quinic acid
Filippa	3.18	11.5	1.8
B 4910	3.19	12.0	3.3
4954	3.21	7.6	6.1
5030	3.20	7.5	6.3
4909	4.11	1.5	4.4
4947	4.31	1.0	3.3
5027	4.22	0.7	5.7
Oberländer Himbeerapfel	3.05	7.0	11.6
James Grieve × *Worcester:*			
James Grieve	3.30	14.0	Trace only
B 5129	3.15	12.3	7.0
5135	3.19	11.2	6.2
5144	3.32	10.8	3.5
5131	4.51	2.0	3.7
5136	4.60	1.5	4.2
5146	3.79	0.5	3.5
Worcester Pearmain	3.40	4.6	3.0

ever, and its spots are usually only detectable in ultra-violet light by means of their blue-white fluorescence.

When chlorogenic acid was present in larger amounts it did not show any relations to the malic acid content. In fact, the variety *Sweet Mc Intosh*, obviously having the "sweet" genotype, had the highest chlorogenic acid content of all varieties studied. The factor responsible for the pronounced variations in malic acid content thus seems to be specific for this acid.

Pleiotropic effects of the variation in malic acid content

As mentioned in the introduction this study was initiated in order to find out whether there was any correlation in acid content between the leaves and the fruits of apples. Although it is planned to describe this more in detail in another connexion (NYBOM, 1959), it may be mentioned already here that there was a certain correlation to be found,

Fig. 3. Fruit samples of 14 seedlings from the population *Filippa*×*Oberländer Himbeerapfel*. The seven samples to the right belong all to the sweet type, while those on the left are normally acid.

the leaf sap of the sweet type usually having a pH of 5.7 compared with 5.5—5.6 for the acid ones.

If such a rather pronounced variation in pH concerned the whole vegetative system of the tree, it might be expected to bring about secondary changes. It may be enough to mention in this connexion the role played by the pH in the expression of anthocyanin coloration in flowers (*cf. e.g.* SCOTT-MONCRIEFF, 1936) and maybe also the correlation between high tissue pH and sulphur shyness in apples and gooseberries found by TURRELL and BENTLEY (1957).

Some of the cross populations were, therefore, studied from various points of view for such pleiotropic effects.

These studies, however, which might have some interest for the plant breeder, are not yet finished. It may be said already now that the "acid" and the "sweet" trees look strikingly alike. Differences in vigour and yield, for instance, have not yet been definitely demonstrated.

Fruit samples from some of the populations were also analysed in greater detail. As the results were rather similar for the various populations only one of them will be presented, namely, the data from 16 seedlings after *Jonathan*×*Cox's Pomona* in Table 4.

TABLE 4. *Fruit characters of some "acid" and "sweet" seedlings after the cross Jonathan×Cox's Pomona.*

Seedling No.	Fruit weight gr.	Ground colour	Red colour %	Fruit firmness	Vitamin C mg %	Tannins ‰	Sucrose %	Glucose %	Fructose %	Total sugar %	Malic acid ‰	Free acid ‰	pH	Dry matter %	Viscosity secs	Colour E_{446}	Turbidity
2232 B_2	100	yellow green	15	30	5.9	1.49	1.3	1.6	5.5	8.4	13.5	8.0	3.40	12.3	24.4	.114	8
2342	90	cream	70	24	2.5	1.33	2.9	1.4	6.5	10.8	21.0	8.6	3.42	11.5	23.5	.186	3
2356	60	yellow green	50	27	1.8	1.48	0.8	2.0	4.9	7.7	12.5	8.4	3.29	10.1	23.2	.167	11
2357	70	yellow green	50	> 30	4.4	1.93	2.9	1.4	4.5	8.8	12.0	7.6	3.40	11.5	24.1	.068	7
2409	70	cream	40	30	3.0	1.88	1.3	0.7	5.5	7.5	15.0	10.2	3.33	11.6	25.0	.082	13
2432	100	green	10	> 30	3.3	1.80	1.5	0.8	5.1	7 4	13.5	10.6	3.18	11.5	24.1	.098	4
2446	90	yellow	25	21	1.3	1.21	1.3	1.2	5.3	7.8	12.0	9.7	3.27	11.5	24.6	.172	28
2459	80	yellow green	35	29	2.7	1.75	2.1	1.4	5.5	9.0	14.5	10.0	3.31	11.5	24.0	.077	15
Average acid	83		41	28	3.1	1.61	1.8	1.3	5.4	8.4	14.3	9.1	3.32	11.5	24.1	0.121	12
2233	70	cream	8	20	5.8	1.44	1.1	1.6	5.7	8.4	4.0	2.7	4.11	12.8	26.3	.133	4
2344	120	cream	40	20	0.7	1.41	2.1	1.1	6.3	9.5	4.0	2.2	4.18	12.9	27.8	.442	29
2347	90	yellow	50	21	0.9	1.78	1.5	1.9	4.5	7.9	3.2	1.7	4.33	13.3	28.5	.750	65
2355	90	cream	30	19	0.9	1.40	1.5	2.0	5.7	9.2	3.0	1.8	4.38	12.7	28.7	.416	42
2407	100	yellow	40	22	0.8	1.64	1.5	1.0	5.1	7.6	3.5	2.1	4.17	13.4	29.3	.455	58
2433	90	yellow green	20	30	3.4	1.84	1.3	0.9	6.3	8.5	3.4	2.1	4.15	12.0	25.0	.120	37
2441	100	cream	40	29	0.5	1.53	1.3	1.2	5.5	8.0	2.8	1.7	4.28	11.5	26.6	1.010	60
2461	70	green	40	22	0.4	2.56	2.1	3.0	5.9	11.0	3.2	2.5	4.00	13.9	30.0	.715	46
Average sweet	91		34	23	1.7		1.6	1.6	5.6	8.8	3.4	2.1	4.20	12.8	27.8	0.505	43

Most of the characters studied were very much alike in the two groups, *e.g.* fruit weight, firmness and colour. The vitamin C-content was considerably higher in the acid group. The variation was, however, always great. Sometimes sweet fruits had a rather high vitamin content, which is pleasing for the plant breeder to know.

The sugar content was on an average somewhat higher in the sweet apples, and the same was true for the tannin content (bitter taste, increased colour of the juice). The viscosity and turbidity were likewise higher in the sweet fruits. Except for the increased yellow-brown colour (higher oxidase activity at the high pH), however, none of these characters were strikingly different for the two groups. Except for the minor trends mentioned, one may say that just as for the tree characters they are astonishingly alike.

The occurrence of sweet and acid apples among the cultivated varieties

It was soon realized that these sweet segregates corresponded in fenotype and most certainly also in genotype to the so-called "sweet apples" occurring only occasionally among the cultivated varieties. They are characterized by a very flat and usually non-aromatic flavour. The presence of acid is hardly perceptible at all, and to most people they appear completely worthless from the point of view of quality. They have no commercial importance and are probably mostly kept because of their curiosity, although very rarely they may be preferred to common apples by persons claiming to be super-sensitive to malic acid.

These sweet varieties are mostly found among early summer varieties where their low acidity permits them to be eaten earlier than if they had been of the acid type. The English variety *Laxton's Early Crimson*, one of the earliest in fact, is such an example. The same is true of the Finnish *Sugar-Miron* and the Esthonian *Pear-apple from Tallin*. In the United States there have been marketed fairly recently some new varieties of this kind, namely, *Sweet Delicious*, *Sweet Mc Intosh* and *Newfane*.

In all, we have in the variety collection at Balsgård 8 varieties which have been found to be sweet apples. None have any importance in practical fruit growing.

The pH distribution of these sweets together with 125 other varieties

TABLE 5. *The distribution according to pH of dessert or culinary varieties of apples, cider varieties of apples and perry varieties of pears.*

pH :	<2.8	2.8—3.0	3.0—3.2	3.2—3.4	3.4—3.6	3.6—3.8	3.8—4.0	4.0—4.2	4.2—4.4	4.4—4.6	4.6—4.8	4.8—5.0	5.0—6.0	>6.0
Number of varieties of culinary and dessert apples		8	69	44	4	1	1	3		2			1	
Number of varieties of cider apples			5	20	11	5	27	23	7					
Number of varieties of perry pears				3	17	23	17	4	1	1				

The data for the cider and perry varieties are quoted from the paper of PHILLIPS, POLLARD and WHITING, 1956.

chosen at random is shown in Table 5. Evidently the same distribution is found as in Table 1. It should be pointed out, however, that in Table 5 the sweet group is over-represented, as they probably constitute all sweet varieties among well over 400 varieties, polyploids and sports not included.

Even though these sweet apples are of minor importance among the dessert and culinary varieties, they obviously play a great role in the cider industry. In Table 5 are also included the pH data for cider apples from the paper of PHILLIPS, POLLARD and WHITING (1956). These data are of great interest in this connexion. Again we have the same bimodal distribution with one group around pH=3.2, and one about or over 4.

As is well known to the cider maker there are two types of cider apples, the acid or "sharp" type and the "sweet". It is now obvious that these two types correspond to the acid and sweet segregates described here, and that they, consequently, differ from each other mainly due to one genetic factor.

It is at the same time interesting and disappointing to see that although the pH-range around 3.6—3.8 seems to be preferred in the cider industry, this value, unfortunately, falls between both genotypes. In practice, therefore, one often has to produce this pH by mixing sharp and sweet varieties with each other.

It is also very interesting to note that these two types are not to be found among the pears. Their pH-distribution is quite different, the peak of the curve just corresponding to the interval in the case of the apples. Obviously the malic acid metabolism of the apples and pears is fundamentally different.

Notes on the segregation of anthocyanin content in some cross combinations

In this connexion the author would like to report some observations on the inheritance of anthocyanin, *i.e.* of red fruit colour, in some cross combinations. As mentioned in the introduction, LEWIS and CRANE (1938) showed that the intense red coloration of *Malus Niedzwetskyana* seems to be due to a single dominant gene. At the same time several modifiers seem to be at work, and as far as the more normal variation in red colour among the cultivated apples is concerned, one has not been able to interprete its inheritance in terms of single factors. However, in two crosses, where the parents are rather alike and in one of these closely related, the presence or absence of anthocyanin seems to have a simple background.

In the combination *Yellow Transparente*×*Early McIntosh*, for instance, there were 20 seedlings of which 10 were quite green (or yellow) as the mother variety. The other 10 seedlings contained a varying amount of anthocyanin. *Early McIntosh* is a daughter variety of *Yellow Transparente*. In a similar combination between *Yellow Transparente* and the early red apple *Roter Ananasapfel* there were 77 seedlings, of which 39 were quite as devoid of anthocyanin as the mother variety and 38 contained various amounts of it. These results seem to indicate that both *Early McIntosh* and *Roter Ananasapfel* are heterozygous for a key gene for anthocyanin, whereas *Yellow Transparente* would appear to lack this gene. Moreover, there are obviously other factors modifying the effect of this key gene.

In other cross combinations there are clearly several anthocyanin factors at work. In the cross *Yellow Transparente*×*Worcester Pearmain*, for instance, there were almost no seedlings quite devoid of red colour. In this case 17 seedlings had 0—10 % red stripes; 5 had about half-red fruits, and 6 had 70—90 % red colour. The analysis of the individual anthocyanins by means of paper chromatography might perhaps shed some light on the genetic relationships in such cases.

In the above-mentioned combination *Yellow Transparente*×*Roter Ananasapfel* the acid content was studied at the same time as the colour. The segregation with regard to both characters was as follows:

Acid, red	28	seedlings
acid, green	33	,,
sweet, red	10	,,
sweet, green	6	,,

An analysis for independent segregation gave $\chi^2=1.40$; $0.3>p>0.2$. Thus, the two factors are obviously not linked with each other.

DISCUSSION

It is well known that the total acid content of apples is built up during the summer when the fruits increase in size. Towards the end of the summer and later, as the ripening processes proceed, much of the acid is again broken down. Therefore, certain varieties with a rather sweet flavour when eaten during the autumn or winter may have had quite a high acid content during the summer. The Swedish local variety *Oretorp*, for instance, is unfortunately worthless from the point of view of quality because of its flat taste when it is ripe in February—March. In spite of this, it had the highest acid content of all varieties studied, namely 21.1 ‰, during the last week of July.

In several papers dealing with breeding experiments in apples the inheritance of flavour and acidity has been reported on. In all these cases, however, the acidity has been determined by taste at the time when the apples have been of prime eating ripeness. It has therefore not been possible to reveal the true distribution into acid and sweet types, nor to obtain any regular segregation ratios. The reason why the analysis in the present case was carried out during the summer, was that the correlation between leaf and fruit acidity may then be expected to be most conspicuous.

Among the earlier authors, WELLINGTON (1924) seems to have been closest to the solution of the inheritance of the sweet apples. He concludes that a sweet flavour behaves as a recessive character. He also finds that certain varieties seem to be homozygous for acidity and mentions, curiously enough, one of the homozygous varieties found also by the present author, namely, *Boiken*. In addition, he considers the varieties *Duchess* and *Montgomery* to be homozygous acid.

CRANE and LAWRENCE, 1933, also show the distribution of the seedlings into various classes of acidity after a great many crosses. They have, however, also taken their records when "the fruits are at their best", which is of course most reasonable from a plant breeding point of view. Thus, they have found "an almost insensible gradation from one extreme to the other". From their table one might, however, infer that the varieties *Royal Jubilee* and *Lane's Prince Albert* are probably homozygous acid, whereas the approximately 20 other varieties studied all seem to be heterozygous.

As to the interpretation of the segregation ratios presented in this paper, the first small samples left perhaps some doubt. Although the present writer is not inclined to agree completely with earlier authors who conclude that "inheritance in apples is polysomic", some of these deviations from a 3 : 1 segregation might rather be interpreted as tetrasomic ratios. However, the later counts presented in Table 2 together with other general considerations, *e.g.*, the regular bimodal distribution also found among the cider apples, rather favour the conclusion that the low malic acid content of the so-called "sweet apples" is determined by one commonly occurring, recessive, major gene, inherited in a normal disomic way.

As long as no positive evidence has been presented, there seems to be nothing to warrant the rather common assumption that polysomic inheritance should be typical for this genetically probably very old and cytologically rather stable group of plants, the subfamily *Pomoideae*.

That most characters hitherto studied in the fruit trees are polygenically inherited seems, on the other hand, to be clear. The same is undoubtedly also true for the total variation in acidity. It is evident from the differences in pH distribution among different cross populations that there are several genes acting on the pH of the fruit. Some families have most of the acid seedlings in the region 3.2—3.4, others in the region 3.0—3.2. Such a difference is easily ascertained by taste (*cf. e.g.* NYBOM, 1958). In the sweet group also there is a considerable variation.

In the case of the cider apples, selection should be expected to favour the intermediate acidities. As is evident from Table 5, however, it has not been possible to bridge the gap between the two types. The use of both sharp and sweet types as parents for the production of new varieties has probably counteracted a systematic accumulation of modifiers. For purposeful breeding it should therefore be better to cross within each group only and to make selection for low and high acidity, respectively.

For the breeding of new dessert, and still less for culinary, varieties the sweet genotype obviously has very little interest as an acid-modifying factor, provided that the dominance of the acid allele is complete. Although the present evidence does not entirely rule out the possibility that the sweet allele has a moderating effect in single doses, this effect is probably not very great. The homozygous varieties, *Signe Tillisch*

and *Åkerö*, for instance, are not markedly more acid than the heterozygous ones.

It is also, of course, possible that the sweet allele in single doses has some beneficial effect on the individuals. This might then explain why this gene is so strikingly common. Of the varieties used in the crosses described here, 18 varieties have been heterozygous compared with only 3 homozygous ones. This is an excess of heterozygotes compared with the number expected according to the allelic frequencies 0.57 for A and 0.43 for a. Also from the works of WELLINGTON, CRANE and LAWRENCE, *cf. lit.cit.*, one may infer that the heterozygous varieties are astonishingly common.

As the sweet type has probably been used only very rarely in breeding work one may wonder why the recessive allele has not been eradicated. If the mutation frequency from A to a were high enough to explain its wide occurrence one should expect sweet sports to be fairly common, *i.e.* Aa → aa. No such mutations are recorded, however, as far as the author knows. On the other hand, a comparatively large number of cases have been reported both in animals and plants where recessive genes, without apparently beneficial effect when homozygous, have still been kept in a high frequency in the population owing to some selective advantage of the gene when heterozygous. WILLIAMS and BROWN (1956), for instance, have recently reported such phenomena in the cultivated sweet cherry. This might then explain why the sweet factor is so common in the apples. Another, though more trivial, explanation would be that the sweet cider apples have acted as a reservoir for this factor. The differences in general characters between cider apples and dessert varieties are, however, too large to make this explanation probable.

The wide spread of the *sweet* factor in apples, also among varieties which seemingly are fairly distantly related to each other, indicates that it may be of very old origin. Further studies on the genetics and physiology of malic acid formation may perhaps give some information on the descent and evolution of cultivated pome fruits. In this connexion it is of interest to note that the wild-growing *Malus Niedzwetskyana* (or *M. pumila var. niedzwetskyana* Dieck, *cf.* HENNING, 1947) is of the sweet type according to CRANE and LAWRENCE (1933). Our *"niedzwetskyana"* at Balsgård is obviously not pure, as it is both acid and heterozygous for the *purple anthocyanin* factor.

SUMMARY

The cultivated apples may be divided into two groups with regard to their content of malic acid, namely, an acid or sub-acid group with a pH between 3 and 3.4, and a sweet group with the pH about or over 4. Most varieties, when ripe on the tree, belong to the first group. The second group contains the so-called "sweet apples", having a very sweet or flat flavour.

These sweet types usually constitute a worthless by-product in the breeding work with apples. They are, however, of great commercial importance in the cider industry.

It appears from the study of 21 different cross combinations that this sweet flavour is determined by one, commonly occurring, recessive gene. The extensive spread of this gene — 80—90 % of all varieties seem to contain it — in spite of its relative unimportance in dessert-apple growing, indicates that it is retained in the population due to some selective advantage in the heterozygous condition.

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THE MUTAGENIC EFFECTS OF IONIZING RADIATIONS AND REACTIVE ETHYLENE DERIVATIVES IN BARLEY

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INTRODUCTION

IN two previous papers (EHRENBERG and GUSTAFSSON, 1957; EHRENBERG *et al.*, 1958) it was shown that ethylene oxide, $CH_2—CH_2$ (O), and ethylene imine, $CH_2—CH_2$ (NH), exert a profound influence with regard to chromosome-conditioned sterility and chlorophyll mutation in barley. Ethylene oxide causes mutation rates, calculated per spike progeny, around the upper limit ever reached with X-rays or neutrons. Ethylene imine is even more effective. Rates two or three times higher than those obtained with ionizing radiations were achieved. In addition, these mutagenic chemicals are of special interest since their mutation spectra, with regard to types of chlorophyll mutation, differ from those of ionizing radiations (*cf.* WETTSTEIN *et al.*, 1958). The differences are evident, too, with regard to the mutual proportions of mutation rates and first generation sterility.

In this paper data obtained from the second field generation will be discussed in order to elucidate whether the previous results from greenhouse tests of chlorophyll mutation are valid for viable mutations, too, especially those of potential value in plant breeding, including the so-called erectoides mutations.

The material used in the various treatments consisted of dormant or presoaked seeds of the Svalöf barley variety "Bonus". The types of treatment are briefly presented in Table 1. Further data on the chemical treatments are to be found in the papers mentioned above. The X-irradiation was carried out at the Genetics Department of the Forest Research Institute (175 kV, filtration 2 mm Al, 1000 r/min.) and the

neutron irradiation in the Stockholm reactor R-1 (the vertical central channel). At a reactor effect of 2 kW, and with 1 cm lead shielding the dose rates were 150 rep/min. of fast neutrons plus about 60 rep/min. of γ-radiation. Doses were measured by means of chemical dosimetry and calculated by integration over the whole neutron spectrum (*cf.* EHRENBERG and SAELAND, 1954 a, b).

The seed was treated immediately before sowing in the spring of 1957. In all kinds of treatment the first generation (X_1),[1] as well as the second generation (X_2), were grown under identical conditions. This permits a fair comparison of the effects of the four mutagenic agents discussed in this paper.

Greenhouse tests were performed at Svalöf and Lund in the winter season of 1957/58. Spikes of X_1 plants were laid in sand at $+10$ to $+15^\circ$ C and the chlorophyll mutations were recorded in the seedling stage. The field experiments were carried out at Hjerupslund, a substation of the Swedish Seed Association (Svalöf). Here seeds of the material studied were sown according to the routine methods used in mutation work for many years, partially in cooperation with the Barley Department of the Seed Association.

GREENHOUSE TESTS OF CHLOROPHYLL MUTATION

The material is presented in Table 1. Except the controls 3 470 plant progenies and 15 812 spike progenies have been examined. In all, 829 chlorophyll mutations were recorded in the treated series and ten mutations in the controls.

(1) The table shows that the rates of chlorophyll mutations induced by X-rays and neutrons reach the same upper limit, *viz.* 7—10 per cent, calculated with respect to spike progenies. At equivalent doses neutrons are much more effective than X-rays in causing mutation. The data indicate an approximate value of 20 for the ratio of effectiveness (*cf.* EHRENBERG and NYBOM, 1954, p. 407).

(2) Ethylene oxide varies considerably in effect, depending on the exterior conditions of treatment. In this set of experiments treatments

[1] Since X-radiation was the first type of mutagenic agent ever used, we prefer to denote the first generation obtained after the treatment of seeds, whether by X-rays, neutrons or mutagenic chemicals, as the X_1 generation, and the progeny raised from the X_1 plants as the X_2 generation. This is not only for historical but also for practical reasons, since numerous previous publications have used this method of denomination, independent of the type of mutagenic agent actually applied.

at low temperature (+5° C) resulted in a high survival and low mutation rates. Treatments of dry as well as soaked seeds at room temperature, on the other hand, produced mutation rates at the maximum level of the ionizing radiations — rates around 6 per cent — confirming the data published in 1957. The temperature effect on the mutation rate corresponds to a Q_{10} amounting to 4 (± 1). The high value is probably conditioned by a temperature influence on the reaction rate, as well as on the diffusion.

(3) The ethylene imine series have been discussed by EHRENBERG *et al.* (1958). The mutation rates in these treatments are much higher than those obtained with ionizing radiations, in fact twice or three times as high, reaching values of 25 per cent or more.

(4) The mutation spectra conspicuously vary with ionizing radiations, on one hand, and ethylene compounds, on the other. The following survey summarizes the data:

Treatment	Percentage			Sum of mutants
	Albina	Viridis	Others	
X-rays	40.2	40.2	19.5	87
Neutrons	42.1	41.0	16.9	183
Ethylene oxide	31.2	42.8	26.1	138
Ethylene imine	24.0	51.8	24.2	421

Such a comparison of mutant types, irrespective of dosage, is not entirely correct, since the various mutation types show an individual dose dependance and are eliminated with different rates at high lethality and sterility (EHRENBERG, 1955). However, in this case the lumped data may be compared, since a detail analysis of individual series does not markedly change the proportions.

In the case of X-rays and neutrons the distribution of mutation types is alike, the small deviations being statistically insignificant. The same holds true for the ethylene compounds *inter se*. Although the albinas are, relatively seen, less frequent in the imine than in the oxide series, the difference is within the limits of error (P=0.2). Comparing ionizing radiations with ethylene compounds, we find a striking difference: with two degrees of freedom a χ^2-value of 21.47 corresponds to a P-value of less than 0.001***. With regard to chlorophyll mutations we conclude therefore that the mutation spectra are widely different, ethylene compounds giving rise to rare chlorophyll mutations more frequently than do the ionizing radiations.

TABLE 1. *Chlorophyll mutations in greenhouse tests 1957/58.*

Treatments	No. of plant progenies	No. of spike progenies [1]	No. of mutants			Sum of mutants	Percentage mutations (per spike progeny)	Mutation rate per rep-unit and spike progeny
			Albina	Viridis	Others			
X-rays								
1 000 r	217	871	4	1	3	8	0.9	9.2×10^{-6}
2 000 r	198	850	9	12	5	26	3.1	15.3×10^{-6}
4 000 r	210	847	15	8	6	29	3.4	8.6×10^{-6}
8 000 r	30	207	4	10	—	14	6.8	8.5×10^{-6}
12 000 r	12	151	2	4	2	8	5.3	4.4×10^{-6}
16 000 r	7	22	1	—	1	2	9.1	5.7×10^{-6}
Neutrons								
160 rep	213	881	14	10	5	29	3.3	205.6×10^{-6}
240 rep	209	1 004	17	12	6	35	3.4	145.4×10^{-6}
400 rep	189	791	22	19	9	50	6.3	158.0×10^{-6}
640 rep	180	688	24	34	11	69	10.0	156.7×10^{-6}
Ethylene oxide								
Dry seeds, 10 hours of treatment								
Room temperature								
0.075 % EO-solution	213	992	7	7	1	15	1.5	
0.10 % »	225	1 035	3	5	5	13	1.3	
0.15 % »	209	1 054	22	28	13	63	6.0	
0.20 % »	9	56	—	2	1	3	5.4	
0.30 % »	16	69	—	1	3	4	5.8	
% 5°C								
0.1 % EO-solution	142	570	—	1	2	3	0.5	
0.2 % »	137	949	—	1	—	1	0.1	
0.3 % »	105	595	3	2	1	6	1.0	
0.4 % »	116	483	1	—	2	3	0.6	

0.1 % EO-solution	142	570	—	1	2	3	0.5
0.2 % »	137	949	—	1	—	1	0.1
0.3 % »	105	595	3	2	1	6	1.0
0.4 % »	116	483	1	—	2	3	0.6
0.6 % »	123	472	1	7	2	10	2.1
Soaked seeds (24 hours), 2 hours of treatment							
0.25 % EO-solution	40	286	6	5	6	17	5.9
Ethylene imine							
Dry seeds, 2 hours of treatment							
0.01 % EI-solution	139	497	9	11	6	26	5.2
0.02 % »	143	473	7	34	20	61	12.9
0.04 % »	103	337	18	56	17	91	27.0
0.07 % »	39	205	11	24	13	48	23.4
0.10 % »	5	29	1	3	1	5	17.2
Soaked seeds (24 hours), 2 hours of treatment							
0.05 % EI-solution	104	525	26	47	20	93	17.7
0.10 % »	50	294	15	16	7	38	12.9
0.15 % »	33	237	7	13	8	28	11.8
0.20 % »	38	255	6	10	9	25	9.8
0.25 % »	16	87	1	4	1	6	6.9
Control	1 241	5 741	2	7	1	10	0.11

[1] One spike per investigated plant was used for the field test (see Table 2).

FIELD MATERIAL

The field analyses were concentrated to the following groups of aberration, easily recognized and isolated in the second field generation:

(1) *Chlorophyll mutations.* Generally a classification of type has been carried out in the field, but owing to annual differences in temperature and precipitation the division into subgroups is rather uncertain (NYBOM, 1955). In consequence, the subdivision of chlorophyll mutations in the field will not be discussed here. (2) *Induced sterility.* Two types occur: (a) slight sterility, amounting to 5—10—20 per cent and chiefly depending on chromosome translocation, in the following analysis denoted as "translocation sterility", (b) profound sterility, from 50 per cent and over, generally depending on factor mutation and recessive in character. Type b is not considered in this survey. (3) *Viable mutations,* which comprise erectoids, "bright-greens", and other types of morphological and physiological changes (GUSTAFSSON, 1947). All of them are viable in the homozygous condition, often high-productive. (4) *Dwarfs,* of variable expression and drasticity, including semilethal homozygotes.

The frequencies of aberrant progenies are given in Table 2. A few remarks are necessary for a correct interpretation of the table. At the high rates of translocation sterility obtained in this material, with values up to 40 per cent or more, it often happens that several different cases occur in the offspring of one and the same X_1 plant. In such an instance, where two or more spike progenies from the same X_1 plant show translocation sterility, it cannot — without a careful cytogenetic analysis in later generations — be established whether the aberrant spike progenies represent the same or different cases of sterility. Sometimes the situation is similar for the chlorophyll mutations, which cannot be identified in the field material with the same certainty as can be done under the controlled conditions of greenhouse tests. For these two types of genetic change every spike progeny showing the change in question is tabulated as an individual case of aberration. Since in about 5 per cent of segregating progenies more than one spike of the X_1 plant is heterozygous for the same change, *i.e.* in such cases when two or more spikes originate from the same primordial cell of the treated embryo, this procedure may lead to the repeated enumeration of individual mutation cases. Some instances, on the other hand, escape enumeration when different cases of aberration, in which the aberrations cannot be morphologically distinguished from one another, occur together in the same spike progeny. At a recognized aberration rate of 40 per cent nearly one fifth will consist of two or more cases occurring simultaneously in the same progeny. In the case of viable mutations the tabulation of every aberrant spike progeny as one individual case has not been applied. For instance, if an erectoid mutation, with its characteristic appearance, has been found in two different spike progenies of one single plant offspring, they have been counted as one mutational case, if the segregates were morphologically alike. The dwarf mutations were tabulated in the same manner. In the case of viable mutants and dwarfs, which are easily defined, it rarely

happens that two (or more) spike progenies of the same X_1 plant give rise to aberrations different genetically but indistinguishable phenotypically. This difference in the grouping of the four types of aberration leads to a certain discrepancy, it is true. To make fair conclusions, we have therefore tabulated the material in various ways. The statistical results were the same independent of method of tabulation.

The control material consisted of 1 303 spike progenies. Two chlorophyll mutations were recorded, one of them uncertain. In 24 progenies there appeared one or more partially sterile plants. Some of these cases are probably environmentally conditioned. Both control figures imply overestimates. In large offspring with low mutation rates often slightly deviating plants, damaged in some way, may be classified as chlorophyll mutants. Similarly, individual plants may now and then show a certain sterility, modificative in character. In such instances third generation tests prove definitely whether there is a genotypic change or not. In the case of mutagenic agents with high effects, this difficulty does not arise to the same extent, since often two, three or more mutated individuals are found in the same progeny and the sterile plants occur in twos or threes. A similar difficulty exists, however, with regard to viable mutations with *slight* effects: a somewhat increased earliness, a slightly increased or decreased density of spikes, stiffness, length of straw, etc. Also here a third generation is regularly bred for routine analysis. However, a trained eye and long experience will remarkably often lead to the detection of small deviations, hereditary in character. Such slight changes are not included in Tables 2 and 3. Clear cases only are recorded.

(1) Considering the maximum rates of aberration (Table 2) there are seven X-ray and neutron series producing more than 30 per cent aberrant spike progenies. The maximum is 62 per cent (for X-rays — 8 000 r). With ethylene oxide there are three such series — the maximum rate being 61 per cent. This indicates that with regard to field material ethylene oxide is, in appropriate treatments, as efficient as ionizing radiations. With ethylene imine there are nine series out of ten which have aberration rates above 30 per cent; in three cases the maximum rates exceed 100 per cent. This means that with ethylene imine treatment a level is reached, which has so far not been found when applying ionizing radiations alone. This is, *nota bene*, in the second generation, where only part of the total number of genotypical changes are unveiled owing to the chimaeric structure of the X_1 plants and spikes, as well as the pronounced X_1 sterility. One may, consequently, expect even higher rates in the third generation.

TABLE 2. *Hereditary aberrations in field progenies of seed materials treated with ionizing radiations and ethylene compounds.*

Treatment	No. of plant progenies[1]	No. of spike progenies[1]	No. of spike progenies with				Total no. of aberrant progenies	Percentage aberrant spike progenies	Rate of aberrant progenies per rep-unit and spike progeny
			chloro-phyll mutations	trans-location sterility	viable mutations	dwarfs			
X-rays									
1 000 r	189	416	6	24	5	—	35	8.41	84.1×10^{-6}
2 000 r	93	391	14	60	13	1	85	21.73	108.7×10^{-6}
4 000 r	88	330	23	84	14	6	127	38.48	96.2×10^{-6}
8 000 r	44	149	22	58	8	4	92	61.74	77.2×10^{-6}
12 000 r	12	12	2	5	—	—	7	58.33	48.6×10^{-6}
16 000 r	17	50	5	10	1	1	17	34.00	21.3×10^{-6}
Neutrons									
160 rep	191	546	16	82	4	—	102	18.68	$1\,167.5 \times 10^{-6}$
240 rep	96	374	14	93	9	8	124	33.16	$1\,381.7 \times 10^{-6}$
400 rep	192	476	57	183	17	16	273	57.35	$1\,433.8 \times 10^{-6}$
640 rep	87	238	19	50	4	6	79	33.19	518.6×10^{-6}
Ethylene oxide									
Dry seeds, 10 hours of treatment									
Room temperature									
0.075 % EO-solution	225	298	11	11	8	1	31	10.40	
0.10 % »	246	320	22	23	9	1	55	17.19	
0.15 % »	223	319	26	34	7	3	70	21.94	
0.20 % »	12	89	12	24	8	2	46	51.69	
0.30 % »	36	143	12	58	11	6	87	60.84	
+ 5°C									
0.1 % EO-solution	170	247	1	—	1	—	2	0.81	
0.2 % »	161	345	1	4	—	—	5	1.45	
0.3 % »	132	230	2	1			3	1.30	
	145	230	8	8	1	—	17	7.42	

+ 5°C								
0.1 % EO-solution	170	247	1	—	1	—	2	0.81
0.2 % »	161	345	1	4	—	—	5	1.45
0.3 % »	132	230	2	1			3	1.30
0.4 % »	145	229	8	8	1	—	17	7.42
0.6 % »	152	243	8	4	7	1	20	8.23
Soaked seeds, 2 hours of treatment, 24 hours of presoaking								
0.25 % EO-solution	69	69	13	7	1	1	22	31.89
36 hours of presoaking								
0.25 % EO-solution	25	25	1	—	2	—	3	12.00
Ethylene imine								
Dry seeds, 2 hours of treatment								
0.01 % EI-solution	177	344	28	32	12	2	74	21.51
0.02 % »	184	292	39	63	14	10	126	43.15
0.04 % »	155	338	86	131	43	21	281	83.13
0.07 % »	42	42	18	17	5	3	43	102.38
0.10 % »	5	5	2	3	—	2	7	140.00
Soaked seeds (24 hours), 2 hours of treatment								
0.05 % EI-solution	128	274	54	95	34	18	201	73.35
0.10 % »	59	115	27	42	19	5	93	80.87
0.15 % »	33	33	5	7	3	2	17	51.52
0.20 % »	38	38	13	15	7	5	40	105.26
0.25 % »	16	16	4	4	4	—	12	75.00
Control	439	1 303	<2	<24	—	—	<26	(<2.0)

[1] Of each plant analysed in the greenhouse test, one spike progeny was tested in the field. Where the number of spike progenies (column 3) exceeds the number of plant progenies, this means that all the spikes of a few additional plants were included in the field test.

(2) Calculated per rep-unit and spike progeny the highest aberration rate for X-rays amounts to $109 \cdot 10^{-6}$, with an average for all six dosages of $73 \cdot 10^{-6}$. For neutrons the corresponding maximum value is $1\,430 \cdot 10^{-6}$, with an average for all four dosages amounting to $1\,125 \cdot 10^{-6}$. For an equivalent energy dissipation, neutrons are consequently 10—15 times as effective as X-rays with regard to the sum of aberrant fields progenies, a somewhat lower proportion than that obtained for chlorophyll mutations in greenhouse tests.

Noteworthy is the striking decrease in aberration rate per rep-unit characteristic for field material at high dosages of both types of radiation.

Within the ethylene oxide series of dry seeds treated at room temperature, there is an evident dose dependance of total aberration rate. At a temperature of $+5^{\circ}$ C aberration rates are low at small concentrations, increasing abruptly at high concentrations, although in the experiments reported here they never reach the values found at room temperature. Also for dry seeds, treated with ethylene imine, there is an evident dose dependance. For soaked seeds all aberration rates reach an extremely high level approaching 75 to 100 per cent.

In Tables 2 and 3 data are gathered with regard to the four classified types of aberrations. Table 3, in contrast to Table 2, records the *total* number of aberrant spike progenies, with regard to viable mutants and dwarfs as well as chlorophyll mutations and sterility.

(3) The mutual rates of chlorophyll mutation and translocation sterility, calculated from Table 2, will first be considered: X-ray induced sterility is approximately three times as common as X-ray induced chlorophyll mutation. With neutrons the ratio is even higher. With the use of ethylene compounds, on the other hand, translocation sterility is relatively less frequent, the rate being at the same level or a little higher than that of chlorophyll mutation. For all types of mutagenic agents used the rates of translocation sterility, calculated per spike offspring, reach a maximum level of circa 40 per cent. With regard to chlorophyll mutation, the ethylene oxide series reach or surpass the maximum levels of the X-ray and neutron series, which amount to 10 or 15 per cent. For ethylene imine seven out of ten series give higher rates of chlorophyll mutation than the most effective X-ray and neutron treatments, in fact rates reaching 40 per cent.

Hence, we conclude that the ethylene compounds, compared with ionizing radiations, increase the rates of chlorophyll mutation relative to the rates of translocation sterility. This may be taken to imply that

the ethylene compounds certainly are capable of breaking chromosomes (a fact established by parallel cytological analyses). This breaking ability, however, is much less, relatively seen, than their ability of producing gene mutation. Anyhow, ionizing radiations break chromosomes (and cause translocation sterility) to a greater extent relative to other hereditary changes, *e.g.*, chlorophyll mutation, than do the ethylene compounds used in these experiments.

(4) From the point of view of plant breeding (and evolution), the high efficiency of the ethylene compounds in producing viable and high-productive mutants is even more interesting. The highest rates (calculated with regard to spike offspring in Table 2) are 5 per cent in the case of X-rays and 4 per cent with neutrons, but rise to 9 per cent with ethylene oxide and 18 per cent or more in the case of ethylene imine. Using ethylene compounds we may consequently expect that under appropriate conditions at least every tenth to fifth spike progeny will give rise to mutations of potential value to plant breeding or, alternatively, to mutations striking from a morphological point of view.

In the following survey the maximum rates of viable mutations are tabulated together with the corresponding frequencies of translocation sterility. The survey is prepared in accordance with Table 2 (spike offspring).

The figures show that for the same extent of translocation sterility the frequency of viable mutants obtained with ethylene imine is con-

Treatment	Maximum rates of viable mutations	Corresponding rates of translocation sterility
Neutrons, 400 rep	3.6 %	38.4 %
X-rays, 8000 r	5.4 %	38.9 %
» , 4000 r	4.2 %	25.5 %
Ethylene oxide,		
dry seeds, 0.2 %	9.0 %	27.0 %
» » 0.3 %	7.7 %	40.6 %
Ethylene imine,		
soaked seeds, 0.25 %	(25.0 %)	(25.0 %)
» , 0.2 %	18.4 %	39.5 %
» , 0.1 %	16.5 %	36.5 %
» , 0.05 %	12.4 %	34.7 %
dry seeds, 0.04 %	12.7 %	38.8 %
» » , 0.07 %	11.9 %	40.5 %
» » , 0.02 %	4.8 %	21.6 %

TABLE 3. *Distribution of types of aberration in field materials of barley.*

Treatment	Calculated per total aberrant spike offspring					Calculated per total aberrant plant offspring				
	Chloro-phyll mutations	Trans-location sterility	Viable mutations	Dwarfs	Sum of aber-rant spike progenies	Chloro-phyll mutations	Trans-location sterility	Viable mutations	Dwarfs	Sum of aber-rant plant progenies
X-irradiation	19.9 %	64.5 %	12.0 %	3.7 %	383	23.1 %	56.8 %	15.7 %	4.6 %	264
Neutron-irradiation	18.5	69.7	5.8	6.0	585	23.3	62.0	7.8	6.9	434
Ethylene oxide	31.9	47.4	16.1	4.6	367	38.2	37.9	18.8	5.1	293
Ethylene imine	30.4	45.1	16.5	7.9	907	35.5	35.9	19.3	9.3	732

Statistical analysis:

X-rays/neutrons — $\chi^2=14.42$, 3 d.f., $P=0.005^{**}$
ethylene oxide/ethylene imine — $\chi^2=4.62$, » , $P=0.20$

X-rays/ethylene oxide — $\chi^2=22.92$, 3 d.f., $P<0{,}001^{***}$
X-rays/ethylene imine — $\chi^2=42.22$, » , $P<0.001^{***}$

neutrons/ethylene oxide — $\chi^2=54.98$, 3 d.f., $P<0.001^{***}$
neutrons/ethylene imine — $\chi^2=94.31$, » , $P<0.001^{***}$

Statistical analysis:

X-rays/neutrons — $\chi^2=11.48$, 3 d.f., $P=0.01^{**}$
ethylene oxide/ethylene imine — $\chi^2=5.17$, » , $0.2>P>0.1$

X-rays/ethylene oxide — $\chi^2=21.81$, 3 d.f., $P<0.001^{***}$
X-rays/ethylene imine — $\chi^2=36.61$, » , $P<0.001^{***}$

neutrons/ethylene oxide — $\chi^2=50.79$, 3 d.f., $P<0.001^{***}$
neutrons/ethylene imine — $\chi^2=79.26$, » , $P<0.001^{***}$

siderably higher than that found with ionizing radiations: three, four or five times as high. Ethylene oxide, too, produces a high mutation frequency, almost twice that of X-rays and neutrons, in one case combined with a relatively low rate of translocation sterility.

(5) Comparing the mutation spectra of X and neutron irradiation statistically, as illustrated in Table 3, both with regard to plant and spike progenies, we find an evident difference (P is 0.01** or less). This largely depends on the relatively low number of viable mutations in the case of neutrons — 34 cases out of 585 aberrant spike progenies or less than 6 per cent, as against 46 out of 383 aberrant spike progenies or 12 per cent with X-rays.

Ethylene oxide and imine, despite the much higher mutagenicity of the latter compound, give approximately identical mutation spectra ($0.2 > P > 0.1$), and the relative proportions of viable mutations are similar, circa 16 per cent in both cases.

Finally, if we compare the ionizing radiations on one hand with the ethylene compounds on the other, the contrast in the distribution of aberration types becomes very striking. In all types of comparisons — X-rays *contra* ethylene oxide or imine, or neutrons *contra* either compound, whether in plant or spike offspring — there appear P-values for the 2×4-comparisons of less than 0.001***. Brought together into two groups — ionizing radiations *contra* ethylene compounds — considerably lower P-values are obtained.

(6) With regard to special types of viable mutations much cannot be said until the progeny tests have been pursued for another generation and the mutated genes have been localized. As visualized in the following survey and in accordance with previous results (EHRENBERG and NYBOM, 1954; NYBOM, 1956), there is, relatively seen, a fairly high number of erectoid mutants arisen with neutrons.

Treatment	Erectoids	Bright-green	Others	Sum of viable mutants
X-rays	6	1	34	41
Neutrons	7	2	25	34
Ethylene oxide	5	4	46	55
Ethylene imine	19	8	114	141

The highest proportions of erectoid mutants are found after the use of ionizing radiations (21 per cent with neutrons and 15 per cent with X-rays as against 9 and 13 per cent in the case of ethylene compounds). Until the localization of the erectoid mutations has been carried out, no

conclusions as to the locus specificity of the ethylene derivatives is possible.

Here it may be added that, just as in the case of chlorophyll mutations, rare mutation types appear to be more frequent after the use of the chemicals tested than after ionizing radiation. Among the viable, more or less drastic mutants isolated in the ethylene imine series (1958), the following may be listed:

Mutant without auricles		2 cases
„	with accordeon-like rachis	1 case
„	„ lemma-like glumes	1 „
„	„ orange lemma	1 „
„	„ obtuse leaves	1 „
„	„ florets not in alternating but in opposite position	1 „
„	„ extra node below the spike	1 „
„	„ short awns	9 cases
„	six-rowed	2 „
„	densinodosum	1 case
„	triaristatum	1 „
„	uniculmis	2 cases

Some of these mutants are rare, some have not even been detected previously in Swedish radiation experiments. Especially noticeable is the finding of the mutants with orange lemma (gene *o* in linkage group V; SMITH, 1951), with obtuse leaves, without auricles, with opposite florets (the last-mentioned mutant was detected, however, in X-ray material already by FREISLEBEN and LEIN, 1944). This indicates that by using chemical mutagens it may really be possible to induce mutations, not or but rarely realized in radiation experiments (*cf.* FAHMY and FAHMY, 1957).

SUMMARY AND CONCLUSIONS

This paper has confirmed the results presented in previous papers, *viz.* that ethylene compounds are highly mutagenic in barley. Mutation rates, never reached with ionizing radiations, have been obtained. This holds true with regard to lethal and semilethal chlorophyll mutations, as well as for viable mutations. In addition, the mutation spectra obtained by means of the ethylene compounds are definitely different from those obtained with ionizing radiations. In previous publications

(EHRENBERG *et al.*, 1956 a, b) dealing with the effects of chemical mutagens, it was concluded that by the use of specific mutagenic agents gene mutations can be separated from chromosome breakage. Such a possibility is indicated also by the experiments reported here. At the same amount of translocation sterility mutation rates obtained by means of ethylene compounds are distinctly higher than those found with ionizing radiation.

After neutron and X-irradiation the rates of viable mutations approximate 4—5 per cent (lowest with neutrons) but amount to 9 per cent with ethylene oxide and to around 20 per cent or more with ethylene imine. This implies that after appropriate treatments with these compounds at least every tenth or fifth spike progeny will show a viable mutant of potential interest for plant breeding. In future, this will give the plant-breeder enormous possibilities for the induction of productive mutants, especially since he will, we consider, be able to separate gene mutation and chromosome breakage. This conclusion is strengthened by the fact that ionizing radiations and chemical mutagens have different modes of action, depending both on the type of radiation and of chemical compound. Hence, various types of mutagenic agents, ionizing and non-ionizing radiations as well as chemicals, ought to be used for the induction of mutations in practice. Here it is of general interest to point out that the mutation spectra after X-ray and neutron treatments are not entirely identical: in the case of neutrons there is a lower frequency of viable mutants but, relatively seen, a higher frequency of erectoides types. Moreover, the neutron-induced erectoid mutations show a difference as to locus distribution (HAGBERG *et al.*, 1958).

Effects of internal and external mutagens have been considered to take part in natural mutability. A minor proportion of spontaneous mutations is, no doubt, caused by the external sources of radiation or the radium or potassium-40 content of the organism. Other mutations arise due to the temperature-conditioned instability of the genes. Under certain conditions the excrete and secrete products of an organism may raise its natural mutation rate, too. A similar rise is achieved now and then by the action of special mutator factors, causing an instability of the entire germ plasm or of individual genes. In addition, genic and chromosomal instability may appear as the result of a heterozygous condition or a disturbed metabolism in general, *e.g.* a calcium deficiency.

This paper necessitates a discussion of additional possibilities. The high mutagenic efficiency of the epoxides and epimines has, no doubt,

to be ascribed to alkylations of biologically important compounds: proteins (RAPOPORT, 1948 a) or nucleic acids (*cf.* EHRENBERG *et al.*, 1958, and literature quoted there). On the other hand, a certain mutagenic activity of the dialcohol formed when the epoxide is hydrolyzed, ethylene glycol, cannot be excluded. This compound was found by ÖSTERGREN (1944) severely to interfere with mitosis and to cause a profound degree of chromosome stickiness. For *Drosophila* RAPOPORT (1948 b) reported a mutagenic action of the glycol 10—15 times less than that of the epoxide but still evident.

It might be anticipated that epoxides and glycols (or the corresponding imines and amino-alcohols), to the extent they occur in living tissue, might add to the rate of spontaneous mutation. For instance, unsaturated compounds may form epoxides *in vivo*, as has been described for carotenoids, among others some with vitamin-A-activity (*cf.* GOODWIN, 1955). A few vegetable oils normally contain epoxy acids, and it is not impossible that epoxides play a role in various biological oxidations, as well as in the autoxidation of fats. The chromosome breaking ability of fats (SWAMINATHAN and NATARAJAN, 1956) may possibly be attributed to effects produced by reactive oxidized products, like epoxides or hydroperoxides.

Ethylene itself is a common, perhaps necessary metabolite in plants, and it is actually released under certain conditions. It exerts an influence on fruit ripening and on growth. It is not known at present whether, under *in vivo* conditions, ethylene can give rise to its epoxide, ethylene oxide, or whether its glycol is a component in its normal or disturbed metabolism. It seems really important to investigate these questions. With all probability ethylene itself is not mutagenic at low pressure; lack of activity in this respect was at least established in our experiments for its 3-carbon homologue, propylene.

The highly mutagenic efficiency of the epoxide and epimine of ethylene is certainly general in character. It has been established for widely different organisms like *Drosophila* (RAPOPORT, 1948 b; CARDINALI, 1954), *Neurospora* (WESTERGAARD, 1957) and various other microorganisms (ALIKHANYAN *et al.*, 1958), *Hordeum* (this and previous papers), and *Pisum* (BLIXT *et al.*, 1958; GELIN *et al.*, 1959). No doubt, their cytotoxic properties also apply to man. In consequence, the use of ethylene derivatives for various purposes in industry and technology must be put under strict control. The mutagenic and carcinogenic effects of the compounds may even appear to be as great as or greater than those induced by heavy dosages of ionizing radiations. In this connection the

ideas expressed by BOYLAND and WOLF (1950) are interesting, explaining the profound biological effects, notably carcinogenesis, of polycyclic hydrocarbons by their assumed ability to form reactive epoxides (*cf.* HADDOW, 1958).

Addendum: In a recent communication HESLOT *et al.* (Compt. rend. acad. sci. *248*: 729—732, 1959) have shown that ethyl methanesulfonate gives extremely high chlorophyll mutation rates in barley, around 40—50 per cent per spike progeny. Hence this compound is equally or even more efficient than the ethylene imine studied by the present authors.

In principle, a molecule of ethyl methanesulfonate constitutes half a molecule of myleran. In HESLOT's experiments the latter compound gives less than two per cent mutations; in our experiments myleran has yielded, under different conditions, mutation rates up to six per cent. The relatively greater mutagenic efficiency established for the mono-functional methanesulfonate thus strengthens the conclusions drawn from comparisons of mono- and di-epoxides (EHRENBERG and GUSTAFSSON, 1957; *cf.* EHRENBERG *et al.*, 1958) that, although di- and poly-functional alkylating substances are more nucleotoxic (observation of, *e.g.*, growth inhibition), monofunctional agents may be more specifically producing gene mutation.

Certainly, the possibilities to induce high mutation rates by means of chemicals are not exhausted by the ethyleneimines and alkanesulfonic esters hitherto studied.

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THE COMPETITION BETWEEN DIPLOID, TETRAPLOID AND ANEUPLOID RYE

THEORETICAL AND PRACTICAL ASPECTS

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I. INTRODUCTION

ONE of the first artificial autotetraploids to be induced among agricultural plants was the tetraploid rye, described by DORSEY in 1936. Since then, breeding work with tetraploid rye has been carried out by several different institutions. MÜNTZING (1951) has reported in detail on his work, during the late 1930'ies and the 1940'ies, with the production of populations of tetraploid rye and with testing their agricultural value and their quality. The work with these populations has resulted in the marketing of one new variety, "Dubbelstål" (Double Steel). Similar work in Germany has resulted in the variety »Tetra-Petkus». v. SENGBUSCH (1940 and 1941) described the preliminary work, of producing the tetraploid population, and further work with the material has been reported by BLEIER (1950), LAUBE (1950), LÖWENSTEIN (1951) and PLARRE (1954). Also in the Netherlands work has been done with tetraploid rye as described by BREMER and BREMER-REINDERS (1954). Finally, tetraploid rye has interested scientists at several other institutions, *e.g.* ANDERSEN (1956) and MORRISON (1956).

Autotetraploids have been produced in a large number of species with varying success from a practical point of view. According to LEVAN (1948) autopolyploidy promises valuable results in the first hand in *cross-fertilizing* species with a *low chromosome number* and of which man utilizes primarily the *vegetative* parts, *e.g.* root crop and herbage plants. Practically valuable results, however, are not attained merely by the production of a tetraploid population, which is rather to be considered as a new and sometimes valuable raw material for further breeding work.

It is important to know as thoroughly as possible the behaviour and

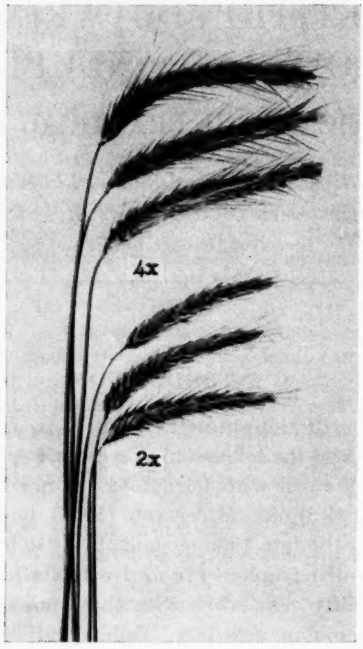


Fig. 1. Some ears of tetraploid and diploid rye of the variety Stål.

reactions of the autotetraploids under the different conditions of breeding, propagation and large scale cultivation. One important problem is the stability of the tetraploid population. Are diploids spontaneously formed and, if so, what are the consequences in practical farming? A related question is: what are the results of intercrossing with, or mechanical admixture of diploids in a tetraploid population? Still another question deals with the frequency of aneuploids and their influence upon the agricultural value of the population.

As already mentioned two varieties of tetraploid rye have been marketed, and the breeding material includes some very promising populations. It may thus be said that rye offers an example of a plant where polyploidy breeding has already given practical results and promises still better ones; in spite of the fact that the grain is the economically most important part of the plant.

Figure 1 shows some ears of the diploid "Stålråg" and the corresponding tetraploid population, the variety "Dubbelstål". These two varieties

have been used in the investigations here reported, which were intended to contribute to the answering of the questions just stated.

II. THE OCCURRENCE OF ANEUPLOIDS

The fertility of the autotetraploids is generally lower than that of the diploids. MÜNTZING (1951, p. 55) reports a seed setting of 76.5 % in diploid rye and of 59.5 % in the corresponding tetraploid. These values are rather low to represent normal conditions. PLARRE (1954) for instance reports the values 84.6 % and 71.3 %, respectively, in a similar material. The climatic conditions influence the degree of fertility. A tetraploid material of spring rye, selected for fertility has a seed setting of about 65 % when grown at Svalöf; the same material grown at Davis, California, showed a seed setting of about 90 % (SUNESON, personal comm.). HÅKANSSON and ELLERSTRÖM (1950) state that "The increased seed sterility of autotetraploid rye compared with that of diploid rye is due to increased failure of fertilization and increased frequency of slow and interrupted development after fertilization. Haplontic sterility seems to be of less importance". According to the same authors, however, as a rule about 20 % of the embryo sacs remain unfertilized.

O'MARA (1943) described meiosis in tetraploid rye and found an irregular distribution of the chromosomes in first anaphase in about 20 % of the pollen mother cells. CHIN (1943) also studied the meiosis of tetraploid rye and found 1.03 multivalents per cell. Similar frequencies of multivalents were reported by PLARRE (1954), BREMER and BREMER-REINDERS (1954) and HILPERT (1957). MÜNTZING (1954) found 3—4 multivalents per cell and in about 60 % of the pollen mother cells there was an irregular distribution of the chromosomes in first anaphase. MORRISON (1956) reported a similarly high frequency of multivalents, on an average about 4 per cell.

A meiosis with such a high frequency of irregularities must result in a fairly high frequency of aneuploids in the progeny. MÜNTZING found 22.7 % aneuploids in an early generation of "Dubbelstål" and O'MARA (1943) found 19 % aneuploids in a freshly produced population of tetraploid rye. In an older population of the German "Tetra Petkus" MORRISON (1956) found 13 % aneuploids.

It is of interest to know if changes in the frequency of aneuploids do occur and also to study the effect of the aneuploids upon the population. Part of the investigation here reported included a study of these problems by a thorough analysis of lots from large scale cultivation of

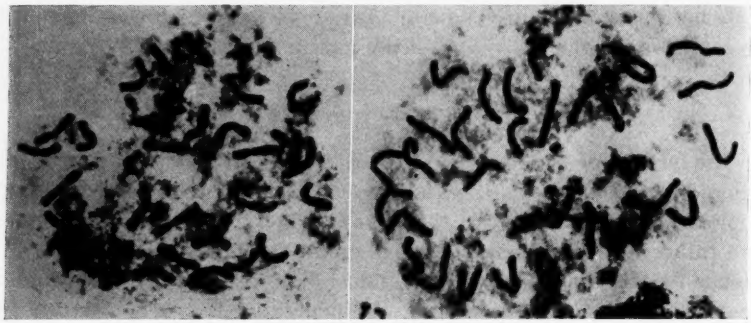


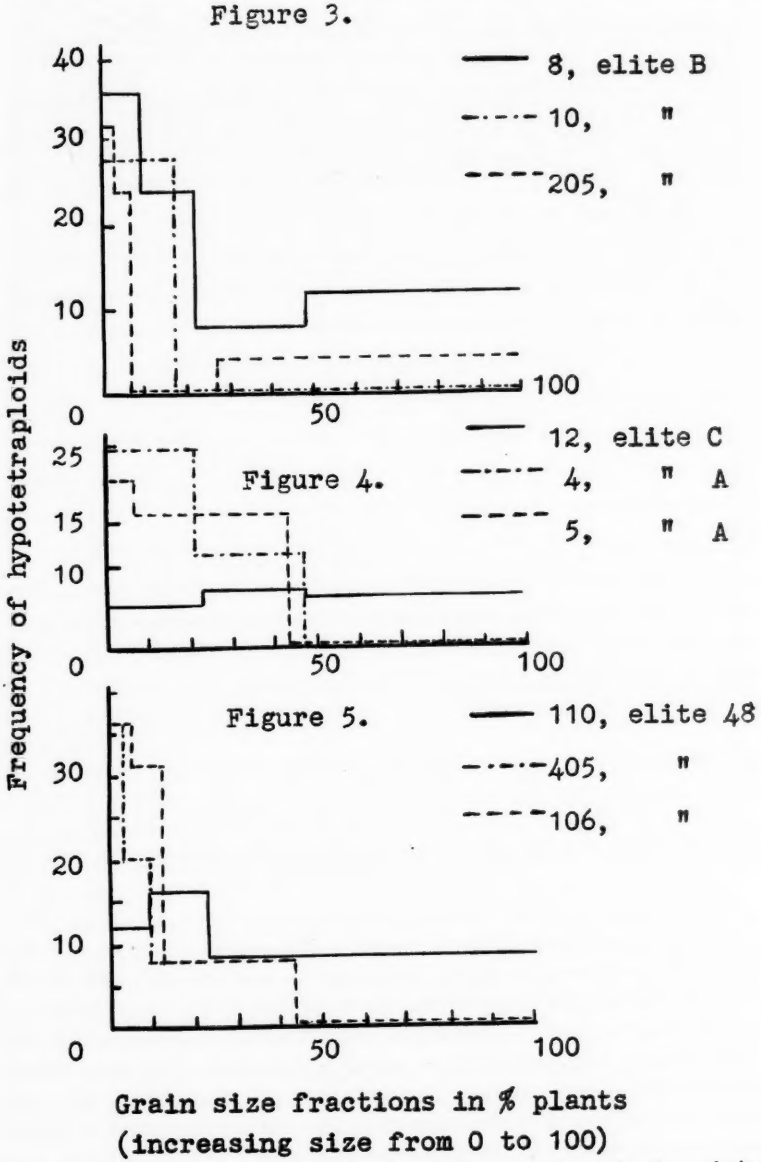
Fig. 2. Two cells of a 29-chromosome plant. Mitosis in root tip squash.

Dubbelstål. This part has already been reported in Swedish (HAGBERG 1953 a); and a summary of the results is included here.

MÜNTZING (1943) showed that no less than 42.5 % of the poorly developed, shrivelled seeds were aneuploids whilst the fraction with well developed seeds included only 16 % aneuploids. Most of the latter were hypertetraploid while most of the aneuploids in the fraction with poorly developed seeds were hypotetraploid. MÜNTZING's data were derived from early generations of Dubbelstål and it should be of interest to compare them with results from an investigation of the same material, more than 10 generations later.

The material for this study consists of official samples for certification drawn in 1952 by a representative of the Central Governmental Seed Testing Station at Stockholm (SCF) and sent to SCF and to Svalöf for routine analysis and chromosome control. The samples were derived from 9 different lots, grown at different localities in South Sweden and were delivered at four different warehouses belonging to the General Swedish Seed Company.

The samples have all been passed through the same set of standard sieves and the fractions have been denoted as follows. I: thickness of grain $<$ 2.00 mm.; II: 2.00—2.25; III: 2.25—2.50; IV: $>$ 2.50 mm. The fractions have been weighed and the weight of 1000 grains determined for each of them; it has thus been possible to determine the weight percentage as well as the number of grains for each of the fractions (Table 1). In each of the fractions the chromosome number has been determined in at least 25 seedlings; thus giving results from at least 100 seedlings from each lot. The calculated means are weighted to allow for the variation in the number of grains per fraction.



Figs. 3—5. The frequency of hypotetraploids in different grain size fractions of nine lots of Dubbelstålråg.

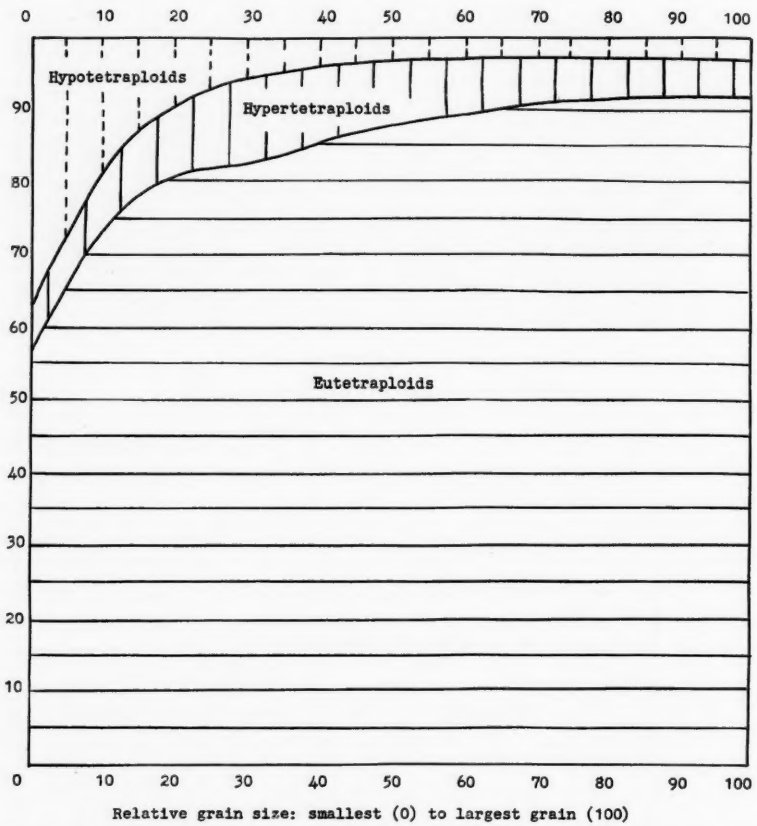


Fig. 6. Distribution of euploids, hypertetraploids and hypotetraploids, in grain size fractions of tetraploid rye.

The chromosomes have been studied in the mitotic metaphase in root tips. After pretreatment with 8-oxyquinoline and staining with orcein, "squash slides" have been made, all according to the "oq-method" of TJIO and LEVAN (1951). In order to determine the chromosome number it has usually been necessary to examine three cells with good "metaphase plates" from each plant, as a rule five such metaphases have been checked. The two metaphases from a 29 chromosome plant in Fig. 2 illustrate the quality of the slides.

The frequency of each chromosome number has been determined for

TABLE 1. *Frequencies of plants with different chromosome numbers in lots of Dubbelstålråg.*

Lot No.	Sieve fraction	Relative weight of fraction (%)	Percentages of seeds	Chromosome number						Total	Percentages of	
				25	26	27	28	29	30		hypoploids	hyperploids
405	I	1	2			9	15	1		25	36	4
	II	7	9			5	18	2		25	20	8
	III	28	31			2	22	1		25	8	4
	IV	64	58				22	2	1	25	0	12
12	I	17	23			4	76			80	5	0
	II	22	24			5	64	1		70	7	2
	III	37	34			3	47	2		52	6	4
	IV	24	19			2	31			33	6	0
Number of chromosomes:			14	21	25	26	27	28	29	30	Total	
Number of seeds in all fractions in all 9 lots:			0	0	1	6	123	847	61	5	1043	

each of the four fractions in each of the nine lots. In tabulating the results plants with numbers below 28 (2n=25, 26, 27) have been pooled as hypotetraploids, while plants with a number higher than 28 (2n=29, 30, 31) have been pooled as hypertetraploids. Among the plants with 28 chromosomes there are a few (*ca.* 1/2 %) which are not true euploids but "double aneuploids" trisomic in one chromosome and pentasomic in another.

The results are shown by the histograms, Figs. 3—5. In these the abscissa represents the grain size, so that between 0 and 10 are the 10 % with the smallest grains, and similarly between 90 and 100 lie the 10 % with the largest grains. The ordinate represents the frequency of hypotetraploids.

All the data concerning the relation between seed size and frequency of hypotetraploids in the different lots, as well as corresponding data for hypertetraploids, are summarized in the graph in Fig. 6. This shows the variation in the frequency of aneuploids with the size of the seed. Among the 100 smallest, of 1000 seeds selected at random, there are on an average 65 euploids, 7 hyperploids and 28 hypoploids. The frequencies of the different types of plants in different size-classes can be estimated directly from the surface circumscribed by the abscissa, ordinate and the respective frequency curves.

The frequencies for two lots are given in detail in Table 1. In the

TABLE 2. *Frequency of aneuploids in different lots of autotetraploid rye.*

Lot No.	Yield dt/ha	1000 grain weight	Sieve fraction, % of seed I	II	III	IV	Frequency of aneuploids % hypo	hyper	total
205	29	48.2	0.9	6.2	20.3	72.6	4.7	15.7	20.4
106	30	44.7	4.8	7.8	29.3	58.1	6.5	9.5	16.0
5	35	43.5	7.2	10.4	26.8	55.6	7.3	9.4	16.7
405	33	42.8	1.5	9.2	30.7	58.6	4.8	9.1	13.9
4	33	42.4	7.5	13.6	26.1	52.8	9.2	5.8	15.0
8	34	40.9	10.1	11.9	28.1	49.9	14.1	4.7	18.8
110	21	40.8	9.0	14.0	32.3	44.7	9.5	7.8	17.3
10	13	37.3	17.5	18.5	34.1	29.9	4.9	1.9	6.8
12	24	34.7	23.0	24.3	34.1	18.6	6.0	1.6	7.6
			Average frequency of aneuploids:				7.44	7.28	14.72

lower part of the table are given the sum frequencies of different chromosome numbers in the whole material. It may first be observed that not a single diploid or triploid has been found among 1,043 seeds, the majority of which belong to the small-grained half of the seed lots, among which the diploids should occur. It seems safe to state that the frequency of diploids in the present material is not more than 0.1 %. This means that in rye there is no spontaneous formation of diploids, or at least the frequency is so low, that the tetraploid population automatically eliminates the diploids, as will be discussed in a later section.

One of the two lots (405) derives from a well developed field giving a good yield; here the grain is of normal tetraploid size. The other lot, with small grains, derives from a field with low yield. The size of the small-grained fraction is very different in the two lots. The larger this fraction, the smaller is the chance to find differences between the fractions in regard to the frequency of aneuploids, as is evident from a comparison between the lots 405 and 12.

Lot 405 shows the typical distribution of the different chromosome numbers, the hypotetraploids being very unevenly distributed among the fractions, while the distribution of hypertetraploids is fairly even between fractions. The distribution of hypotetraploids among fractions in lot 405, deviates significantly from the distribution 1:1:1:1, $\chi^2=11.5$, $P<0.01$. For the total material the corresponding P-value is <0.001, in spite of the fact that lot 12 is a marked exception from the general rule. In regard to the hypertetraploids there is no significant deviation from 1:1:1:1 ($P\sim0.1$).

As is evident also from Table 2 the percentage of seeds within each fraction varies very much between the different lots. Although the average frequency of aneuploids (14.7 %) is lower than the frequency (22.7 %) found by MÜNTZING some ten generations earlier, there is a considerable variation between lots: from 6.8 % to 20.4 %. Thus, closely related populations have a very different frequency of aneuploids. There seems to be a correlation between seed size and frequency of aneuploids; in Table 2 the lots are arranged according to the 1000 grain weight. This may be in agreement with the interesting observation, that the same population of tetraploid spring rye has quite different levels of fertility when grown in California and in Sweden, which is now being more closely studied as to regularity of meiosis and frequency of aneuploids.

Attempts have been made to increase fertility and establish populations with a lower frequency of aneuploids by strong selection for regular distribution of the chromosomes in anaphase I of meiosis. Preliminary data were published by HILPERT (1957). She found a certain effect of selection but the results do not give much hope for rapid progress by this method.

It has been very difficult to obtain a good estimation of the importance of the aneuploids in the population, the main reason being the difficulty to obtain comparable plant material of aneuploids. All aneuploids found in the analysis of chromosome number have been planted as seedlings in pots and later transferred to the field populations. Very often, however, they have not been able to compete with the other plants in the stand. Thus, good measurements have not been obtained. A number of aneuploids have been isolated in a separate group for crossing within the group. The aneuploids were mainly plants with 27 and 29 chromosomes respectively and in addition a few plants with 26 and 30 chromosomes respectively. This group of aneuploids has been compared to a similar group of pure tetraploid plants (euploids) giving a better comparison. The euploid plants were as an average more vigorous and had a higher fertility. Seed set in the euploids was 40.7 per cent compared to 14.9 per cent among corresponding aneuploids. The low values for seed set are due to the relatively restricted number of plants in each of the isolated groups.

Thus, there are so far no certain data concerning the behaviour of aneuploids in rye populations. Hypoploids seem to be less vigorous than hyperploids which is in good agreement with LEVAN's (1942) results on sugar beets and with similar results from other crops. All aneuploids,

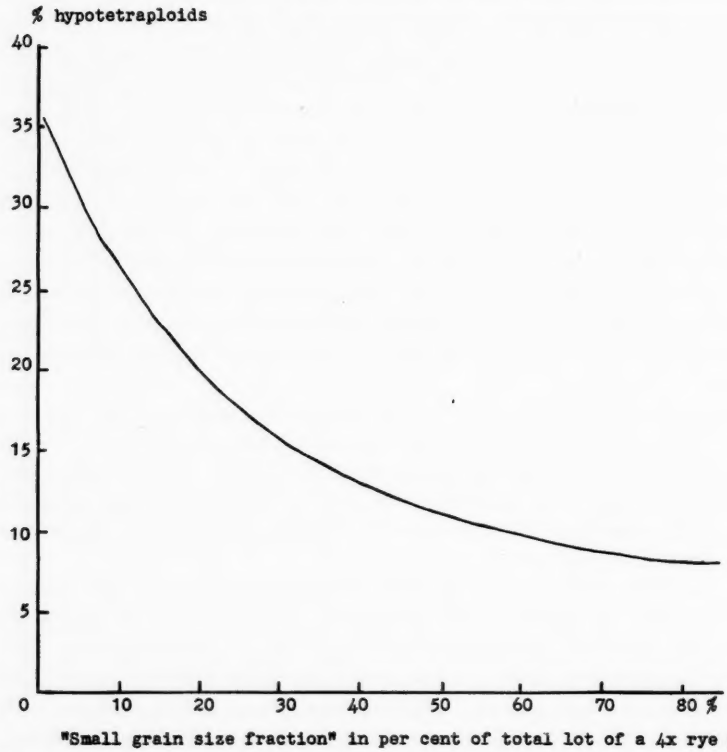


Fig. 7. Frequency of hypotetraploids in the fraction of small grain, if a lot of tetraploid rye is sifted in two fractions. The smaller the fraction of small grains the higher is the frequency of aneuploids.

however, are inferior to the euploids and there is no doubt that the aneuploids and especially hypoploids are a "draw back" in the tetraploid populations. No doubt a reduction in the number of hypotetraploids would be an advantage. This is possible to achieve in most lots by sieving the seed. The graph on Fig. 7 shows the frequency of hypoploids in the small grain fraction passing the sieve of an average lot of tetraploid rye. Thus, if the "small grain fraction" has the size of 10 per cent it contains 26 per cent hypoploids, if it is 20 per cent of the whole lot it contains 20 per cent hypoploids and if 30 per cent is sieved away as the "small grain fraction" this fraction will contain about 15 per cent hypoploids.

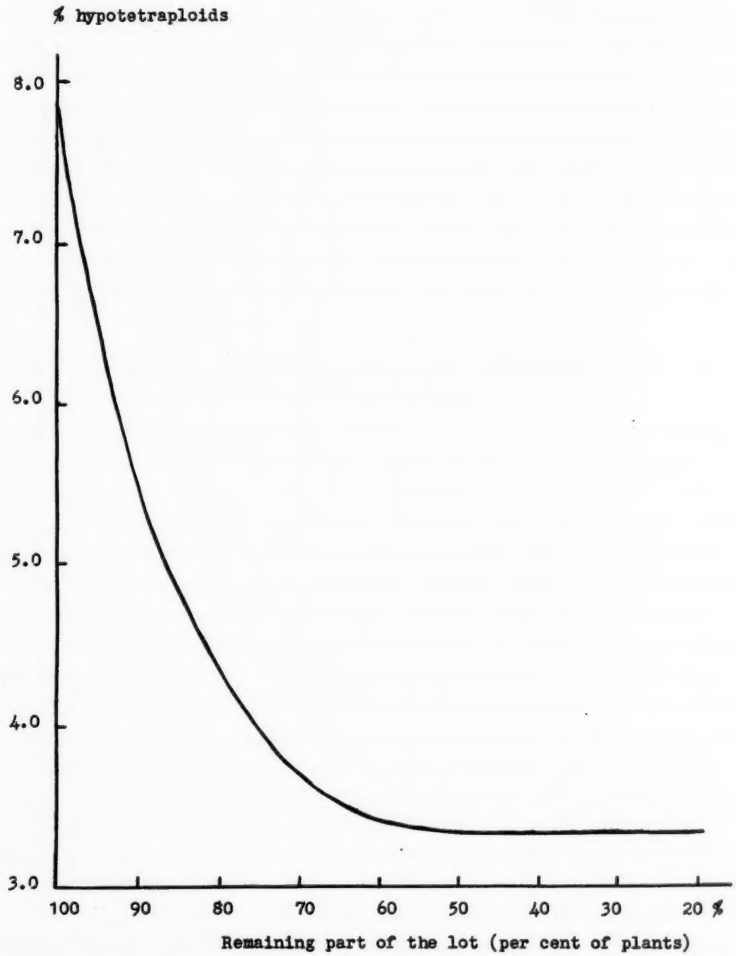


Fig. 8. The frequency of hypotetraploids in the remaining part of the lot when a minor or larger fraction of small grains is sifted from the lot.

It is, however, more important to know the frequency of hypoploids in the "large grain fraction" after sieving an average lot of tetraploid rye. The graph in Fig. 8 gives this information. If a "small grain fraction" of 10 per cent is sifted from the lot, the frequency of hypoploids is decreased from 7.8 to about 5.5 per cent. If 20 per cent is sifted from

the lot, the hypoploid frequency is decreased to about 4.5 per cent. The half of the lot containing the large grains will have a hypoploid frequency of about 3 per cent and about 6 per cent of hyperploids.

Sifting off a 20—25 per cent small grain fraction from a tetraploid population in general considerably decreases the frequency of hypoploids and in addition diploids eventually admixed disappear from the population. It is generally advisable to clean and sieve every lot of tetraploid varieties, which is to be used for seed production. However, it may be pointed out that an extreme sifting — 40—50 per cent or more — may result in a selection not desirable as will be discussed later.

III. THE STERILITY BARRIER BETWEEN DIPLOID AND TETRAPLOID RYE

It is often difficult to cross varieties with different chromosome numbers, especially if they are on a different level of ploidy, and a sterility barrier is found between diploids and their corresponding autotetraploids. This barrier may function in different ways. In some cases the triploid embryos formed abort and none or very few triploid individuals are developed. In other cases the triploids may be vegetatively fully vital but sterile and their few progeny plants have an irregular chromosome number as described by several authors, *e.g.* LEVAN (1942). Examples within the genus BRASSICA, embryologically studied by HÅKANSSON (1956) show a gradation between the two types of barrier. In turnip (*Br. campestris*) it is difficult to obtain triploids, only a few often very small seeds develop into grown triploid plants. In *Br. oleracea*, however, seeds are plentiful in a cross between diploid and tetraploid. These seeds are very small, however, the weight of 1000 grains being 1.2 grams as against 4.1 grams for normal diploids and 5.3 grams for tetraploids (HOWARD, 1939). The same conditions prevail in *Sinapis alba* (OLSSON and RUFELT, 1948).

In beets it is possible to produce triploids on a large scale. They are very vital and in some cases superior to both diploids and tetraploids in regard to yield. So called triploids or polybeets — in fact a mixture of triploids, diploids and tetraploids — of sugar beet are commercially marketed. In several other agricultural plants the conditions are, however, similar to those in turnip — the triploids abort in the embryo stage. This is true in barley (HÅKANSSON, 1953), maize (COOPER, 1951), rye (HÅKANSSON and ELLERSTRÖM, 1950) as well as in alsike clover (ARMSTRONG and ROBERTSSON, 1956) and red clover (JULÉN, 1950).

CHIN (1943) has studied reciprocal crosses between diploid and tetraploid rye. He obtained good seed setting after 4x×2x but no seeds after the reciprocal cross. Unfortunately, the chromosome numbers were not determined in the seeds from the cross 4x×2x but probably several of them were not triploid but tetraploid. ELLERSTRÖM (unpubl., quoted by MÜNTZING 1951) obtained a seed setting of 0.31 % in the cross 2x×4x and of 0.51 % in the reciprocal combination. In all he obtained 8 triploids from 2058 pollinated flowers. In both combinations a considerable number of small, shrivelled and not germinable "seeds" were formed besides a few well developed ones. There is no significant difference between the reciprocals. The poor seed setting is not due to failure of the pollen to fertilize the embryosacks. In his study ELLERSTRÖM also used a mixture of 50 % haploid and 50 % diploid pollen grains. In the combination 2x×"mixed" only diploid progeny was obtained and no shrivelled seeds, while 4x×"mixed" gave 3 triploids, 27 tetraploids and a large number of shrivelled seeds from 520 pollinated flowers.

LÖWENSTEIN (1951) has also studied the sterility barrier between diploid and tetraploid rye, using mixed pollen and has obtained similar results. HÅKANSSON and ELLERSTRÖM (1950) and LÖWENSTEIN (1951) have described in detail the embryo development of diploids, tetraploids and after the reciprocal crosses. The two first mentioned authors state (p. 284): "The very large and almost complete seed sterility after diploid×tetraploid and tetraploid×diploid rye matings is not due to failure of fertilization ... The cause is irregular development and disintegration of the endosperm." Even if the frequency of viable triploids is about the same in both reciprocals it seems as if the triploid embryo develops somewhat further on a tetraploid mother. The endosperm is somewhat better developed before disintegration than in the reciprocal cross. The higher frequency of triploids after 4x×"mixed" than after 2x×"mixed" is probably due to the more rapid growth of haploid pollen and has nothing to do with differences in embryo development.

When tetraploids are used in agriculture it may sometimes be difficult to avoid intercrossing with and admixture of diploids. The sterility barrier between the two levels of ploidy must to some extent serve as a protection for the tetraploid populations, but on the other hand it includes some danger, as was early pointed out by RANDOLPH (1941). The studies reported in the following sections intend to elucidate the effect and consequences of the sterility barrier when tetraploid rye is grown on a commercial scale.

IV. STUDIES ON THE EFFECT OF VICINISM BETWEEN DIPLOID AND TETRAPLOID RYE

Fairly early it became evident that diploid and tetraploid rye could not be compared in the same yield test, the sterility barrier causing severe deprecation of yield, especially in the tetraploid (MÜNTZING, 1948). It is necessary to compare the tetraploids with wheat in one test, the diploids with the same wheat in another test, sufficiently distant but if possible under the same environmental conditions. The necessity to use indirect comparisons between tetraploids and diploids makes it difficult exactly to estimate the yielding capacity of the former. There is another problem, however, which is perhaps even more important: is it at all possible to grow a tetraploid variety of rye in a region with intense cultivation of diploid rye, where the air must be more or less filled with haploid pollen during flowering time? What is the minimum distance between fields of diploid and of tetraploid rye if a deprecation in yield in both of them is to be avoided?

a. Vicinism experiments at Ugerup in 1950—1951

In order to answer the first of these questions a field of about 1 hectare of Dubbelstål was sown in 1949 on the South Swedish experimental farm Ugerup, situated in a region with intense rye cultivation. The nearest diploid field was only 50 metres distant, all other rye in the region was diploid and there must be a rather dense "pollen cloud" in the air during flowering. Nevertheless the seed setting in the tetraploid field was normal along the borders as well as in the centre and the yield was quite satisfactory.

Next year a field of Dubbelstål was sown in the middle of a large field of diploid rye (Petkus II), the results already being presented in Swedish (HAGBERG, 1953 b). At the same time fields of Dubbelstål, about 2 hectares, and diploid rye were grown at the distance of at least 100 metres from one another.

The degree of fertility was determined in samples of about 50 ears each, taken at random in the large field of Dubbelstål and at certain fixed points in the field of Dubbelstål included in diploid rye. Even in the latter case the sample was a random sample of ears growing at or close to the point decided. Figure 9 gives the results of the fertility analysis, each value being the mean of 25 ears, selected at random from the sample. Along the western border 8 samples were taken immediately on each side of the border, the distance between the points being 3 metres.

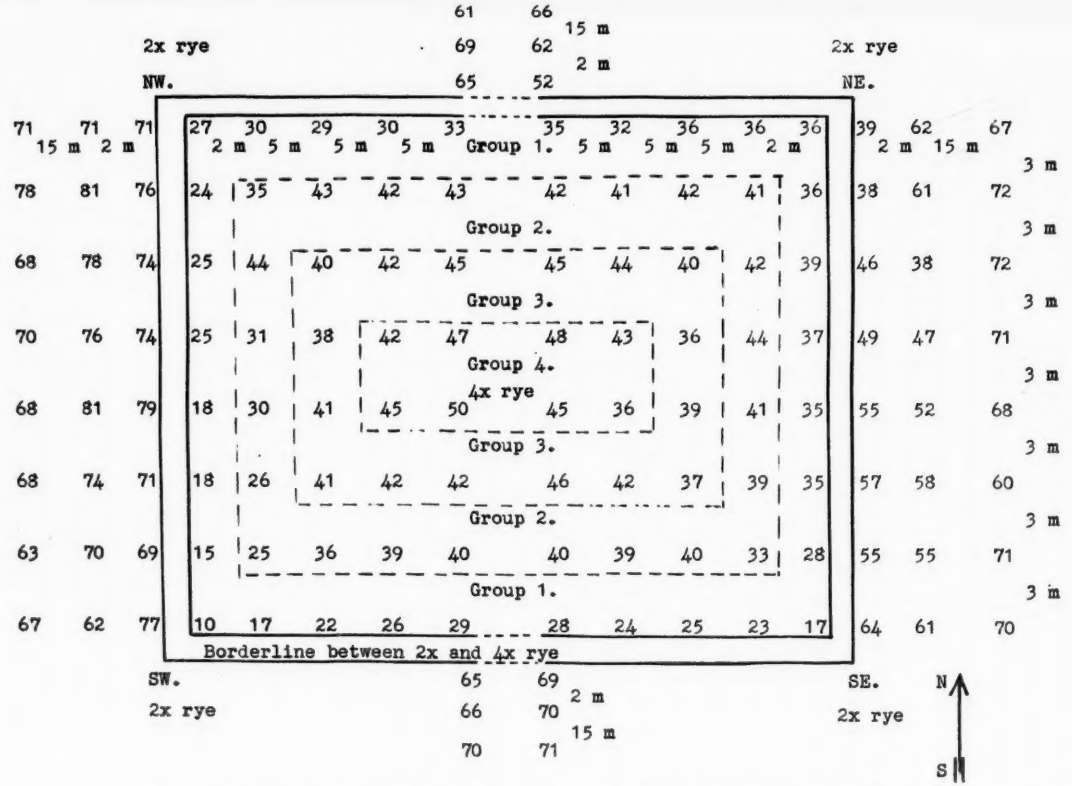


Fig. 9. Seed setting per cent in samples of ears from a field of tetraploid rye, enclosed in a field of diploid rye at Ugerup 1951.

The series was repeated on both sides 2 metres from the border and in the tetraploid again at 7, 12 and 17 metres, in the diploid at 17 metres. A similar series was taken starting from the eastern border, and finally samples were taken in the diploid north and south of the tetraploid.

The seed setting in the comparatively isolated field of Dubbelstål was 60.1 ± 1.01 % while the corresponding diploid field had the unusually low value of 69.0 ± 1.48 %. In the surrounded tetraploid the lowest fertility was found at the southwestern corner with only 10 %. The fertility rose northwards along the western side and eastwards along the southern, with a marked drop at the southeastern corner. The fertility along the northern and eastern sides was again higher with no drop at the northeastern corner. Southwesterly winds were prevailing during flowering. 2—3 metres from the border the seed setting was higher and fairly even except in the southwestern corner. 6—7 metres from the border and further inwards the fertility was fairly even with a mean of 42.3 %. This value is significantly lower than the one found in the "isolated field" which was 60.1 % ($t=13.56$, $P<0.001$).

The samples from the tetraploid field have been primarily grouped into four groups: 1, samples from the border line; 2, samples taken 2—3 metres from the border line; 3, samples taken 6—7 metres from the border; 4, the 8 samples from the centre. There is no significant difference between groups 3 and 4 ($t=1.78$) and in the following comparisons they are pooled. The comparison between group 2 and 3+4 gives $t=3.04$, $P \sim 0.001$. If 2 is compared only with 3 $t=2.26$ and $P<0.05$. The most striking difference is between group 1 and group 2 with $t=6.35$, $P<0.001$.

Within group 1 the samples from different sides of the field have been compared. The western and southern sides are not significantly different ($t=1.64$); the seed setting along the northern and eastern borders is nearly the same, 32.6 and 32.9, respectively. The western and southern borders are significantly different from the eastern and northern ($t=4.41$, $P<0.001$).

Also within group 2 the sides have been compared. There are no significant differences between the southern, eastern and northern sides (mean values 39, 40 and 42, respectively) but the seed setting on the western side is significantly lower ($t=3.46$, $P<0.01$). The samples along the northern and southern sides are taken 3 metres from the border, those in the east and the west only 2 metres, which partly explains the high values, especially those along the southern side.

The average standard error for a difference between two samples is

TABLE 3. *Chromosome numbers in samples from the trial at Ugerup 1951.*

Sample	2n = 14	21	26	27	28	29	30	Total	% 3x	% aneupl.
SW-corner 4x	0	6		1	33	4		44	14	13
All other 4x	0	10	5	27	265	14	5	326	3	16
NE-corner 2x	237	0			0			237	0	0

2.81, a difference of 7.5 % thus gives $P \sim 0.01$. A specially interesting comparison is the one between the corner samples and other samples along the border. Both the southern corners have lower values than the border samples from the middle of the corresponding sides and this "corner effect" is also apparent in the samples next to the corners and in the southwestern corner sample in group 2.

The diploid samples from the western border, which have been "to windward" during flowering, have a significantly higher fertility than those from the eastern border ($t=6.49$, $P<0.001$). The same is true for samples taken 2 metres from the border ($t=5.08$, $P<0.001$). 17 metres from the tetraploid there is no difference between the eastern and the western side of the diploid.

To the west of the tetraploid there is on the whole no significant difference in fertility between samples taken at the border and at 17 metres into the field. The diploid border samples from the north-eastern corner have a significantly lower fertility than those from the middle of the eastern side, the difference to the southeastern corner is 25 %, $t=5.27$, $P<0.001$. The variation between ears is large in the diploids from the eastern border, resulting in high standard errors. It is nevertheless obvious that the variation is continuous from north to south. It might be suspected that the low fertility in some of the diploid samples was due to the occurrence of tetraploids, having been mixed into the diploid by carelessness in sowing. A check of the chromosome number has shown, however, that all the ears in the samples have been diploid, as seen from Table 3.

This table also gives the chromosome numbers in the progeny of the ears sampled in the southwestern corner of the field of tetraploid rye. The fertility of this sample was determined as 10 %. Among seeds formed 14 % were triploids, corresponding to 1.4 % of all flowers. Probably triploid embryos are formed in at least 50—60 % of the flowers and only 2—3 % of these resulted in well developed ripe seed. All data concerning chromosome numbers from the other samples in

the tetraploid field have been summarized in the second line of Table 3. In this material 3 % of all seeds studied were triploid. It is thus evident that if a comparatively small field of tetraploids has been subject to an intense cloud of haploid pollen, there will be a certain low percentage of triploids in the progeny. The results from the sample taken from the most exposed corner of the diploid field show that no triploids have been found when the cross has been made with the diploid as the mother plant.

The seed setting of the diploid rye was fairly low at Ugerup in 1951. MÜNTZING (1951, table 12, page 49) presents data for the seed setting at Svalöf in the years 1944—49, and only in 1948 had the diploid Steel rye a lower value than the one obtained for diploid Petkus II in Ugerup 1951. The value for the "isolated" field of Dubbelstål is also somewhat lower than normal, but the difference between the two levels of ploidy is less than usual. In any case, the "pollen cloud" from the numerous large fields of diploid rye around Ugerup has not in 1950 and 1951 had any important effect upon the fertility of the separate fields of tetraploid rye.

The tetraploid field, surrounded by diploid had, however, if the borders are excluded, about 20 per cent units lower fertility than the separate tetraploid field. In this case the pollen cloud from the large surrounding diploid field has been sufficient to cause this very considerable decrease in seed setting in the tetraploid. The result is a decrease in yield. The yield of the tetraploid was 22 decitons/hectare, that of the surrounding diploid 30 dt/ha; the relative yield of the tetraploid is 73 if the diploid=100. The separate field of tetraploid yielded 42 dt/ha, the comparable diploid field 40 dt/ha. In this case the relative value for the tetraploid is 105. ANDERSEN's (1956) data, from a similar 4x field within a 2x field in 1954, on the whole confirm the results obtained at Ugerup in 1951.

It should be mentioned that one of the neighbours of the experiment farm grew a small field — about 0.25 hectare — of diploid rye close by the "isolated" tetraploid field. This does not seem to have caused any appreciable deprecation of the seed setting in the latter. In this case the tetraploid field was large enough, so that the pollen cloud above it was completely dominated by its own pollen.

The part of the diploid field which was most exposed to pollen from the tetraploid had a considerably decreased seed setting. Tetraploid rye thus may influence the seed setting of diploid rye. This was shown also in the already quoted Table 12 of MÜNTZING (1951). In 1944 and 1945

diploids and tetraploids were included in the same trial. Not only the tetraploids but also the diploids show an abnormally low fertility. LÖWENSTEIN (1951) has sown alternate plots of diploid and tetraploid rye, 2 metres broad and 40 metres long. There was a marked decrease in fertility in both levels of ploidy, this being most marked along the borders. Similar results have been obtained by OLSSON and RUFELT (1948) in an experiment with white mustard. In another experiment LÖWENSTEIN also could demonstrate the effect of prevailing winds during flowering; the decrease in seed setting found by him corresponds fairly well with the Ugerup data.

LÖWENSTEIN (l.c.) also showed that strips of tetraploid rye, growing at different distances from large fields of diploid showed an increase in fertility with increased distance from the diploid. At 100—150 metres from the diploid field the seed setting of the tetraploid was normal. He presented some other data, however, which show a somewhat different tendency. The shortest distance between two large fields of diploid — 10 hectares — and tetraploid — 30 hectares — rye was 80 metres. In the tetraploid field, samples taken from the border and up to a distance of 15 metres into the field, showed a decrease in fertility of not less than 15 %. At 15—30 metres into the field the decrease in fertility was about 10 % and it was still 3 % 30—45 metres from the border. In the diploid field the decrease in fertility was about 10 % at the border, whilst further into the field there was a corresponding smaller, but appreciable decrease.

These last results are important and well worth noting. The data from Ugerup has not shown the possible effect of the pollen cloud on large fields to the same extent. The fertility in the German studies were (in both levels of ploidy) considerably higher than we are accustomed to in southern Sweden. Possibly this is one of the reasons for the discrepancies in the results.

b. Vicinism experiments at Svalöf in 1952—1956

In order to be able to give advice to the farmers with regard to "free position" it is necessary to study the effects of vicinism. The most pertinent question is the distance necessary between fields of diploid and tetraploid in order to avoid losses in either. To get further information about this question a series of experiments were carried out at Svalöf in the years 1952—1955. A number of plots of tetraploid rye, each having an area of 200 sq. metres were sown annually. These plots were sited, at

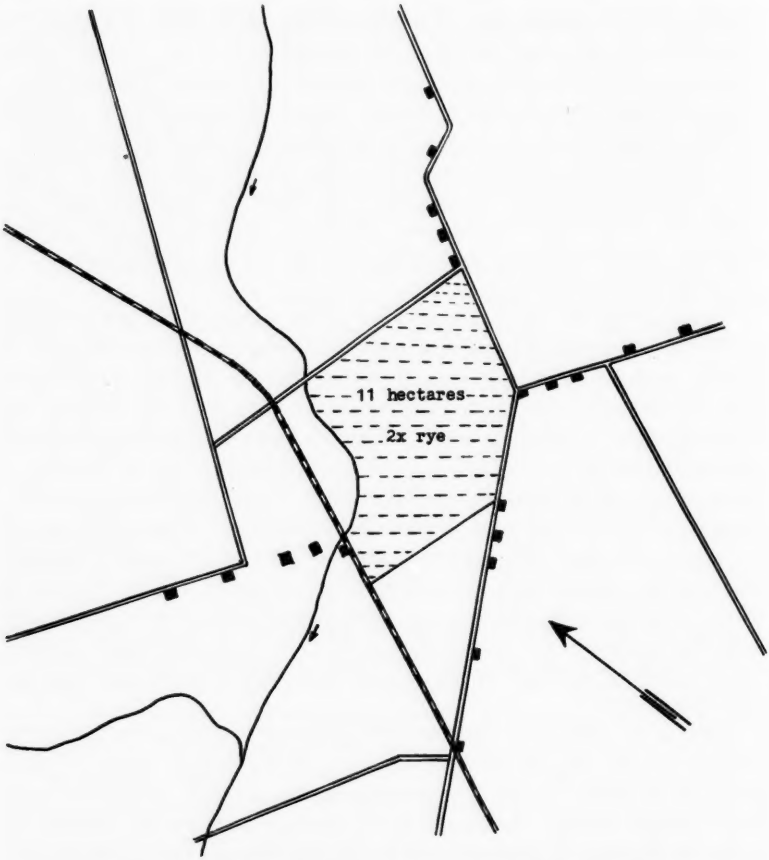


Fig. 10. Map showing the distribution of plots in the trial of 1953—1954. The filled squares represent the 4x plots.

various distances from a centrally situated large field of diploid rye, and in such a manner that they surrounded this field on each of its four sides. In order to avoid as far as possible disturbances to the normal farming procedures it was necessary to place the plots of tetraploid rye on the edges of fields which also gave easy access to roads. As an example of the planning Fig. 10 gives a map of the arrangement in 1953. During flowering careful observations were made concerning strength and direction of winds but also in regard to cloudiness, temperature and

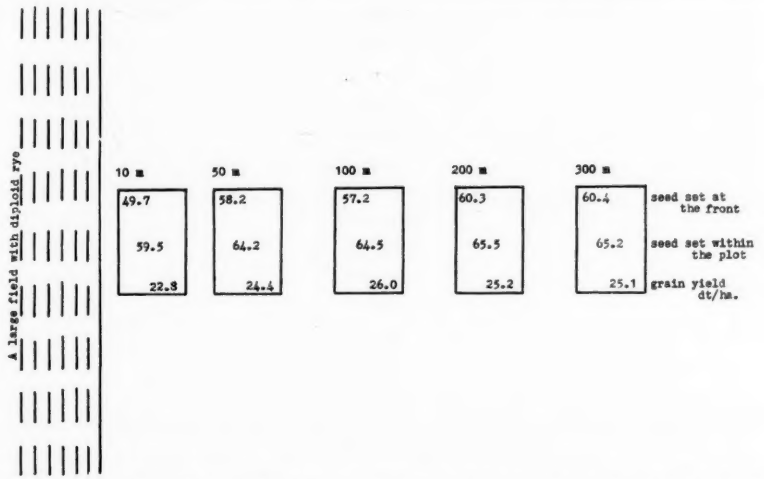


Fig. 11. Five plots of tetraploid rye at different distances from the diploid field, representing three year trials each with the five plots in the four cardinal points.

precipitation. At harvest ear samples were taken, similar to those in Ugerup. Ten samples were taken along the border, of the tetraploid plot, closest to the central diploid field, ten other were taken in the centre of each plot.

Each plot has been harvested and threshed separately and the yield of grain and straw determined. It has been impossible to avoid differences between the plots, even within the same "row". Each year the experiment has covered an area of about 1 square km and it has been impossible to avoid considerable edaphic differences. In spite of this and of a fairly low average yield the data obtained are sufficient as a basis for a discussion of the effects of vicinism.

The most important data are summarized in the diagram, Fig. 11. It presents average seed setting and grain yield in the plots of tetraploid rye at different distances from the large field of diploid. All values given are means of equivalent values from the four "rows" of plots during all three years. Each value, thus, is the mean of 12 primary values; these again, with regard to fertility, are each founded on determinations carried out on 250 ears.

In the plots 10 metres from the diploid field the fertility is 49.7 % along the border nearest the diploid material. In the centre of these plots the fertility is 59.5 %, a surprisingly high value. In the plots at

North
57.3
54.9
52.9
47.4
41.1
West 66.9 65.1 65.2 62.2 48.4 [2x] 49.5 54.7 54.5 57.9 57.5 East
65.5
66.0
66.6
65.6
67.2
South

Fig. 12. Seed set in the front of the tetraploid plots towards the central field (2x rye) in the trial 1953—54.

50 and 100 metres the border fertility is 57—58 %, in the centre it is 64—65 %, thus reaching normal fertility for tetraploid rye under the conditions prevailing. The plots at 200 and 300 metres have a fertility of about 65 % in the centre, and about 60 % at the border. The low fertility at the border in these cases is probably not due to influence from the diploid field, but rather to the fact that conditions for pollination are always better within a field than at the border. As to yield there is no increase when the distance becomes greater than 100 metres. If the mean yield of the 100, 200 and 300 m plots is counted as 100 the 10 m plots have a relative value of 89.9, the corresponding value for the 50 m plots being 97.2.

The summarized data of Fig. 11 indicates that a distance of 100 metres between a diploid and a tetraploid field should be sufficient, under the conditions prevailing in these experiments to prevent an appreciable decrease in fertility. It is obvious, however, that the prevailing winds during flowering will influence the results. Fig. 12 gives the seed setting along the border of the different plots in the experiment of 1954. During the flowering period of 1954 the prevailing wind was southerly with deviations to southwest and southeast; even without meterological observations this would have been evident from the data. All the plots to the south have a normal fertility, while especially those to the north show a marked decrease; the same is true of the eastern "row" and to a smaller extent also of the western. The effect on the

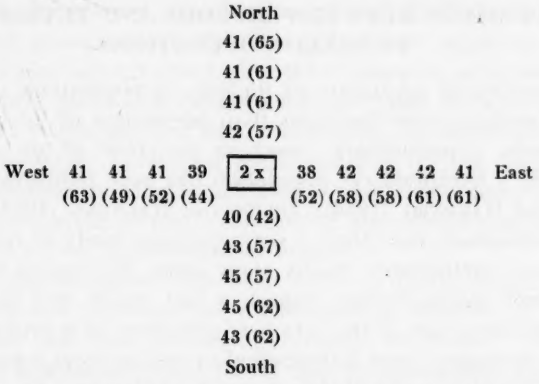


Fig. 13. The percentage of grain of the total yield (straw+grain) in the different tetraploid plots in the trial 1952—53. The average seed set in "front samples" from respective plots are given within brackets.

fertility in the centre of the plots is much less marked as is also the influence on yield.

In a rye population there is a considerable variation in earliness, and the flowering days of different ears vary considerably. In 1955 ears were marked which flowered on the very first day, when the wind was westerly to northwesterly. In the 10 m plot to the east the seed setting among these ears was 34.8 %, while the corresponding ears in the three other 10 m plots had a setting of 63.0, 61.4 and 67.9, respectively. Similar results were obtained from ears flowering during the following day with similar wind conditions. On the fifth day of flowering the wind was from the south to the south-southeast. Ears flowering on this day had a setting of 37.3 % in the northern 10 m plots, 55.3 in the western and 69.7 in the southern plot. Two days later the wind was again westerly; ears flowering this day in the eastern plot had a setting of 53.0 %.

These data show the effect of wind direction more strongly than the annual averages. These only show the effect of prevailing winds, which again is of the greatest importance in regions where winds of a certain direction usually prevail at the time of year when the rye is flowering. In such regions it should be avoided to place a field of tetraploid rye leewards of a diploid one; or at least in such cases the distance between the fields should be considerably more than the 100 metres otherwise suggested as sufficiently safe.

V. COMPETITION BETWEEN DIPLOID AND TETRAPLOID RYE IN MIXED POPULATIONS

The mechanical admixture of diploids in populations of autotetraploids is perhaps more insidious than the danger of intercrossing between fields. A preliminary report on the effect of an admixture of diploids in a tetraploid rye population has been published by ELLERSTRÖM and HAGBERG (1954); JULÉN and HAGBERG (1954) have published preliminary data from a corresponding study in red clover. Already these preliminary results show great differences between different plant species in this respect, a fact which will be referred to during the discussion of the data here presented. It is evident, however, that it is necessary, from a theoretical as well as from a practical viewpoint, to elucidate in detail the effects of admixtures.

The experiments with mixtures of diploid and tetraploid rye were started in the autumn of 1952, when eight plots of 400 square metres each, were sown at 70—100 metres from each other. In seven of these plots diploid rye was mixed into tetraploid, the percentages of admixture being 1/2, 1, 2, 4, 8, 16 and 32 %, respectively; the eighth plot was sown with pure tetraploid. In mixing the two types germination and grain weight were taken into consideration and the mixing was done very carefully to ascertain an even distribution of diploids in the plots.

In 1953 plots were sown with pure tetraploid and with admixtures of 1, 4, 16 and 50 % of diploids and finally with seed from the plots which in 1952—53 had carried admixtures of 1, 4, 8, 16 and 32 % diploids. In 1954 new mixed populations were sown with 4, 32, 40 and 50 % of diploids as well as plots with pure tetraploid and pure diploid populations. Finally, in 1955 plots were again sown with 4, 16, 32 and 50 % of diploids admixed, with pure tetraploid and diploid seed and one plot with alternating diploid and tetraploid strips, each strip being 20 metres long and 0.65 metres wide. In each of the mixed plots diploid seed was sown at 20 specially marked places; 1 plant was left at each place, which was observed, especially at heading, flowering and ripening. In the plots with pure stands triploid and aneuploid seedlings were planted, but these were so weak that they could not be utilized for more detailed determinations.

From each of the different mixed plots five samples were taken by digging out the plants. Five such samples were taken in November, in April, and again at harvest, each sample consisting of 200 plants, selected at random. By counts and measurements on these plants it was

possible to decide whether the frequency of diploids is changed during the vegetative phase and whether the diploids are suppressed by the tetraploids or *vice versa*. Table 4 gives the frequencies of diploids found in some of these plots. The variation between years is mainly random and the mean values give the best information. On the whole there is no marked selection. There is a weak tendency for the frequency of diploids to decrease but the significance is rather low. Some measurements have been made on plants sampled in the autumn and spring but they have not shown any definite differences.

Extensive measurements have been taken on plants samples at the time of harvest and the results are summarized in Tables 5—9. The first of these gives the data concerning tillering. MÜNTZING (1951) and SCHILDT and ÅKERBERG (1951) found that in pure stands diploid rye

TABLE 4. *Frequency of diploids in mixed populations of diploid and tetraploid rye.*

In the seed (September)	Frequency of diploids in the field during the vegetation period		
	before winter (November)	after winter (April)	at harvest (August)
	1953—54		
4 %	7.4	3.0	3.1
16 %	7.8	14.0	18.7
50 %	51.1	50.0	51.8
	1954—55		
4 %	1.0	3.6	2.8
32 %	28.0	23.0	30.0
40 %	28.0	30.0	33.0
50 %	41.0	40.0	39.0
	1955—56		
4 %	5.5	4.5	2.8
16 %	31.6	15.2	17.9
32 %	40.5	35.3	32.6
50.%	60.7	53.5	51.0
	Average:		
4 %	4.5	3.7	2.9
16 %	19.7	14.6	18.3
32 %	34.3	29.2	31.3
40 %	28.0	30.0	33.0
50 %	50.9	48.5	47.9

TABLE 5. *Tillering in plant samples taken at harvest time in the mixed rye populations.*

Year	Frequency of diploids in the population	4 %		16 %		32 %		40 %		50 %		Pure stand	
	Level of ploidy	2x	4x	2x	4x	2x	4x	2x	4x	2x	4x	2x	4x
1954	number of tillers	1.00	1.03	1.05	1.04					1.03	1.05		
	significance of. diff.	0		0						0			
	no. of plants	19	593	115	500					380	353		
1955	number of tillers	1.71	1.44			1.73	1.63	1.37	1.29	1.31	1.25	1.34	1.24
	significance of diff.	0				0		0		x		x	
	number of plants	17	584			168	392	191	383	380	568		
1956	number ot tillers	1.64	1.45	2.06	1.51	1.49	1.57			1.39	1.36		
	significance of diff.	0		xxx		0				0			
	number of plants	11	382	65	299	129	292			236	227		
	Average relative mean 2x=100		90		82		98		94		98		93

Significance: xxx means $P < 0.001$, xx $P < 0.01$, x means $P < 0.05$. 0 means no significance.

TABLE 6. *Length of straw in plant samples taken at harvest time in the mixed rye populations.*

(Compare Table 5)

	4 %		16 %		32 %		40 %		50 %		Pure stand	
	2x	4x	2x	4x	2x	4x	2x	4x	2x	4x	2x	4x
1954	131	132	111	127					116	127		
	0		xxx						xxx			
1955	89	39			93	98	103	113	95	101	100	107
	0				xxx		xxx		xxx		xxx	
1956	117	127	118	115	107	109			109	115		
	x		0		0				xxx			
Average relative mean 2x=100		104		106		103		110		107		107

tillers more than tetraploid rye. The same is true in the different mixed stands. Only one of the individual comparisons gives significance but when all results are summarized the significance is high. The relationship with regard to tillering is not changed with the variation of the frequency of diploids.

The straw (Table 6) of tetraploid rye is taller than that of the diploid. MÜNTZING obtained the same result in pure stands. In a German study on different material (PLARRE 1954) the diploid was tallest. When the frequency of diploids is low the difference in straw length is less than when the frequency is high. The few diploids in the former case grow taller under influence of the surrounding tall tetraploids (cf. HAGBERG, 1952, pp. 42—43, the analysis of competition between different strains of *Galeopsis*).

Concerning length of ear (Table 7) the same holds true as in regard to straw length. The tetraploid ear is longer than the diploid, but in mixed populations with a low frequency of diploids their ears become comparatively longer, nearly as long as those of the tetraploid. The number of spikelets per ear (Table 8) is somewhat less in the tetraploid than in the diploid; the relation seems independent of the frequency of diploids.

It is obviously of primary interest to study the seed setting and to compare, in this respect, the pure stands with the diploids and tetra-

TABLE 7. *Length of ear in plant samples taken at harvest time in the mixed rye populations.*

(Compare Table 5)

	4 %		16 %		32 %		40 %		50 %		Pure stand	
	2x	4x	2x	4x	2x	4x	2x	4x	2x	4x	2x	4x
1954	7.5	8.0	6.3	7.6					6.4	7.8		
	0		xxx						xxx			
1955	7.0	6.4			5.6	6.7	6.8	7.7	5.3	6.1	5.3	6.3
	0				xxx		xxx		xxx		xxx	
1956	7.1	7.4	6.8	6.9	5.4	5.9			5.7	6.7		
	0		0		x				xxx			
Average relative mean 2x=100	101		111		114		113		119		118	

ploids in mixed populations. The results from pure stands in 1955 and 1956 show that the seed setting of the tetraploid is 10—15 % lower than that of the diploid (Table 9). An admixture of 4 % diploids means a decrease, in the fertility of the tetraploids, of at least 10 %. The diploids are rather sterile (fertility about 20 %) which must be attributed to the

TABLE 8. *Number of spikelets.*

(Compare Table 5)

	4 %		16 %		32 %		40 %		50 %		Pure stand	
	2x	4x	2x	4x	2x	4x	2x	4x	2x	4x	2x	4x
1954	25.3	24.6	23.1	23.4					23.6	24.0		
	0		0						0			
1955	20.9	18.7			20.7	19.8	24.8	23.5	19.6	19.1	19.5	18.4
	0				0		x		0		0	
1956	25.7	22.0	23.9	20.7	21.0	18.2			21.2	20.3		
	0		xxx		xxx				0			
Average relative mean 2x=100	91		94		91		95		98		94	

TABLE 9. *Fertility, per cent of seed set.*
(Compare Table 5)

	4 %		16 %		32 %		40 %		50 %		Pure stand	
	2x	4x	2x	4x	2x	4x	2x	4x	2x	4x	2x	4x
1954	20.0	56.3	25.3	46.2					41.8	28.1		
	xxx		xxx						xxx			
1955	24.1	60.4			41.8	43.5	41.1	38.9	49.6	38.0	81.1	66.0
	xxx				0		0		xxx		xxx	
1956	18.0	55.5	32.8	43.1	46.9	37.6			54.9	31.0	81.1	70.0
	xxx		xxx		xxx				xxx		xxx	

low frequency of haploid pollen. Fig. 14 shows some diploid ears, from different mixed populations, clearly showing the increase in fertility with increased percentage of diploids. At the same time the fertility of the tetraploid decreases. There seems to be an equilibrium somewhere between 32 % and 40 % of diploids, where both levels of ploidy have the same fertility, about 40—45 %. As expected, when the diploids reach a frequency of 50 %, they surpass the tetraploids, the fertility of the former being about 50 % while that of the latter has decreased to 30—35 %.

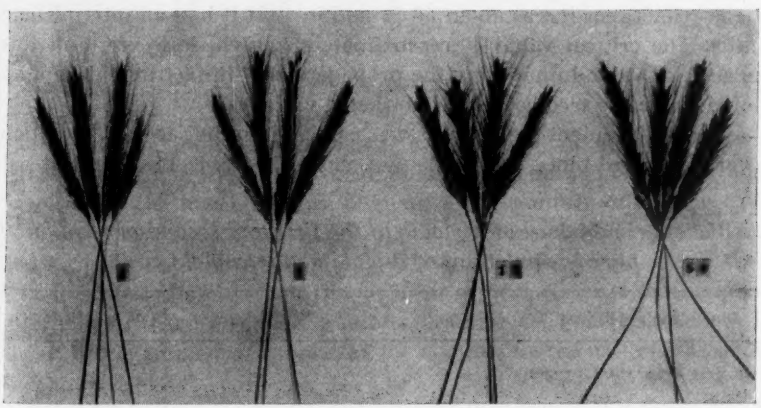


Fig. 14. Diploid ears from mixed populations of diploid and tetraploid rye with frequencies of diploids of 1, 4, 16 and 50 % respectively.

TABLE 10. *Frequency of diploids in the first generation progeny from mixed populations of tetraploid and diploid rye.*

Diploids in original seed:	0	0.5	1	2	4	8	16	32	40	50 %
Diploids in the progeny:										
1952—53	0	0	0	0	0	0.6	2.3	19.2	—	—
1953—54	0	—	0	—	0	—	1.3	—	—	54.2
1954—55	0	—	—	—	0.2	—	—	29.9	35.6	49.8
1955—56	0	—	—	—	0.3	—	9.3	36.4	—	65.0

During the vegetative phase there is no marked selection. Possibly there is a small decrease of diploids but this is counteracted by their better tillering ability and the higher number of spikelets per ear. With regard to fertility there is strong selection — the direction depending upon the relative frequencies of the two levels of ploidy. There is an equilibrium at about 32—40 % diploids, and if there are no differences in germination such populations can be expected to retain this proportion generation after generation. The frequencies of diploids in seedlings from the harvest of the different mixed plots are given in Table 10. The results agree fairly well with the ones already discussed. There is a critical value near 40 %, below which the diploids will decrease, and the tetraploid population is then "self-cleaning"; above the critical value the population will gradually change to pure diploid. The critical value varies somewhat with the years. In 1956 it was obviously below 32 %, the previous year it was about 50 % and in 1954 it had an intermediate value. The critical value is approximately known, however, and this must be of value in handling the propagation of the tetraploid population and for the seed control of marketed varieties.

In one experiment the analysis was carried into the second generation after the mixed plots, the results are shown in Table 11. The decrease

TABLE 11. *Frequency of diploids in the first and second generation of mixed populations of diploid and tetraploid rye.*

Diploids mixed in seed 1952	0 %	1 %	4 %	8 %	16 %	32 %
in first generation progeny 1953	0	0	0	0.6	2.3	19.2
in second generation progeny 1954	0	0	0	0	0.6	17.1

in frequencies of diploids has continued, although in this case not as rapidly as in the former cases.

As in other parts of this work the chromosome numbers have been determined on material fractioned by sieving. Table 12 may serve as an example as to the methods used in handling the material. The meaning of the different columns is explained on page 372. As expected the diploids occur mainly in the small-grained fraction:

	Diploids	Tetraploids	Total
small-grained portion	64	168	232
large-grained portion	3	42	45
Total	67	210	277

$\chi^2 = 7.27$, $P < 0.01$

The frequency of aneuploids follows the scheme already discussed. There is no triploid in Table 12, and among the 846 seedlings analyzed in 1953 there was in all one triploid.

TABLE 12. *Chromosome numbers in grains from the plot VIII 1953, the seed of which contained 32 % of diploids.*

Sieve fraction	% of grains	Grain size 1000 gw.	Chromosome number 14	21	26	27	28	29	Total	% 2x
1.75	4.0	25.8	24	0	2	14	49	2	91	26.4
2.00	4.6	29.0	10	0	2	2	27	3	44	22.7
2.25	36.0	38.0	30	0	2	6	58	1	97	30.9
2.50	55.4	54.5	3	0	0	3	37	2	45	6.7

In the population here discussed the frequency of diploid ears was 27 %; the heavier tillering of the diploid has not been sufficient to compensate for the lower number of plants. The number of spikelets per ear was 26.33 in the diploid and 26.37 in the tetraploid, *i.e.* in this case the same in both levels of ploidy. The seed setting was significantly lower in the diploid, 30.9 % as against 38.0 % in the tetraploid ($P < 0.001$). It is not possible here to describe each of the analyses in detail; the example given must suffice to illustrate the material reported in Tables 4—12.

It is of interest to note the grain size of the diploid and tetraploid in the different mixtures and its dependence upon the seed setting. MÜNT-

TABLE 13. *Analysis of variance of the grain size data from 1956, discussed in the text.*

Cause of variation	DF.	Sum of squares	Variance	Quotient	P
Between plots	7	5467.20	781.03	119.37	< 0.001
» G: A—F	1	2806.72	2806.72	428.97	< 0.001
» (E—F) : (A—D)	1	554.31	554.31	84.72	< 0.001
» E : F_{2x}—F_{4x}	1	340.03	340.03	51.97	< 0.001
» F_{2x} : F_{4x}	1	1276.90	1276.90	195.16	< 0.001
» A, B, C, D	3	489.24	163.08	24.92	< 0.001
» A,B,C : D	1	424.01	424.01	64.80	< 0.001
» (A—C) : B	1	0.21	0.21	1	—
» A : C	1	65.02	65.02	9.94	≈ 0.001
Within plots	152	994.55	6.54	—	—
Total	159	6461.75	—	—	—

ZING has given the 1000-grain weight of the tetraploid as about 50 % higher than that of the diploid. In 1956 the 1000-grain weight of the diploid in pure stand (plot G) was 29.3 grams, that of the corresponding tetraploid 43.7 (plot A). With an increasing admixture of diploids the average 1000-grain weight at first increases; in plot B with 4 % diploid it is 45.1 g., in plot C with 16 % it is 46.3 g. Both these values are significantly higher than that for pure tetraploid. Above 16 %, however, the average 1000-grain weight of the plot decreases, in plot D with 32 % diploids it was 38.7 and in plot E with 50 % only 36.3. Plot F was sown with alternating strips of diploid and tetraploid. The 1000-grain weight in the tetraploid strips was 48.6 g., in the diploid strips 35.7 g. The statistical analysis of these data is given in Table 13; plot F_{4x}—4x and F_{2x}—2x have been treated as two different plots. With a decrease in fertility in the mixtures the 1000-grain weight of the mixture increases. This is true for low frequencies of diploids. When this frequency reaches between 16 and 32 % the fertility of the diploid is fairly good and the small grain of the diploids decreases the average value of the mixture. The diagram in Fig. 15 shows the relation between 1000-grain weight and seed setting in the different populations. This relation is the result of three factors, one represented by the line A—F_{4x} and another by the line F_{2x}—G. Both these factors act in parallel while the third factor — the relative frequencies of the ploids — acts in an opposite direction. The first of the lines mentioned gives the relation between seed setting and grain size in the tetraploid, and the second one is the same relation

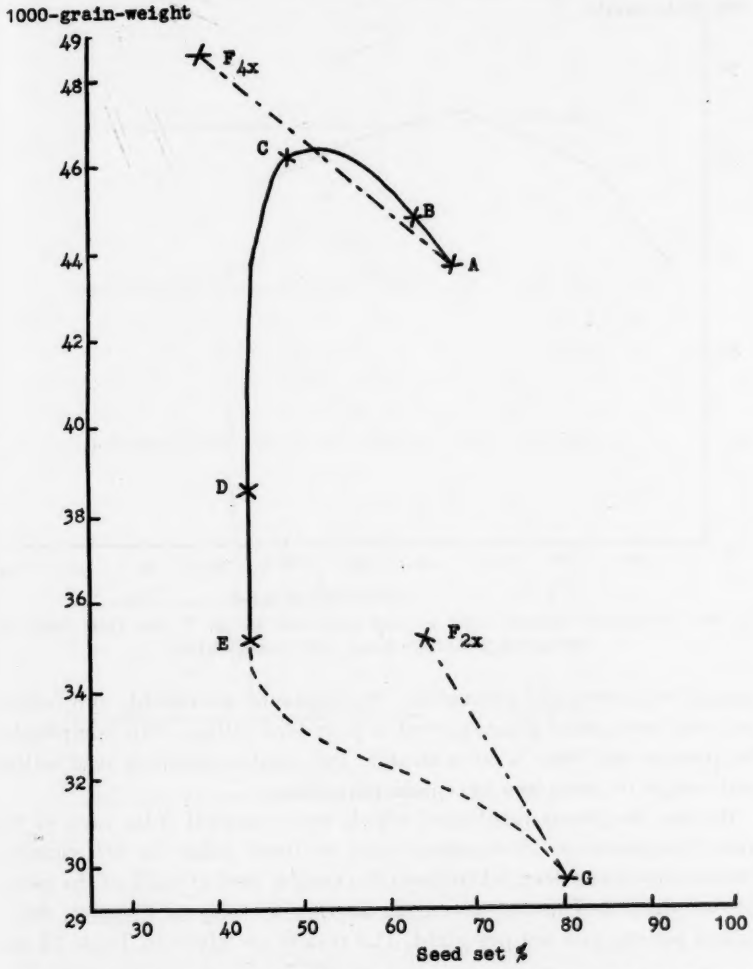


Fig. 15. Correlation between seed set and grain size in the 1956 plots of diploid-tetraploid mixtures. The dotted line, E—G, is hypothetical.

in the diploid. In both cases a reduction in fertility results in larger grains. This means that the lower the frequency of diploids in a mainly tetraploid population the larger will be their grains and the more difficult it will be to eliminate them by sieving. As seen from Fig. 16 there is a fourth factor influencing the relation between seed setting and grain

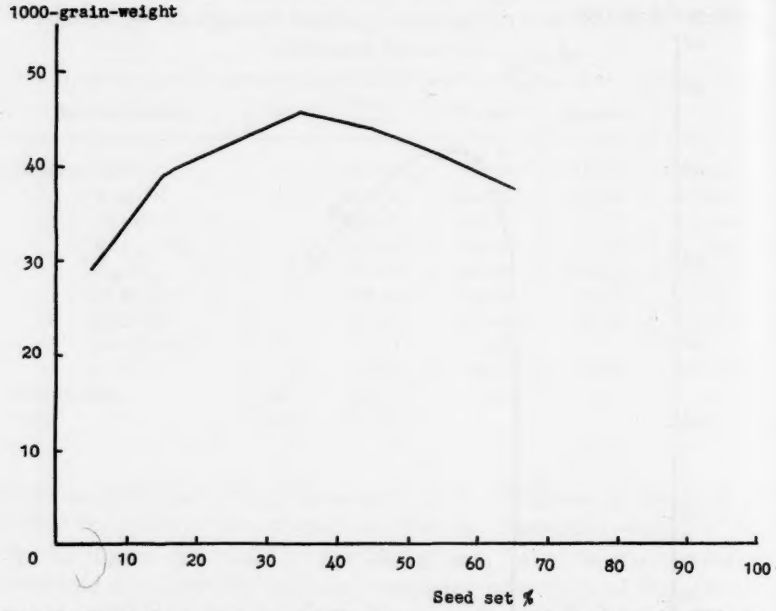


Fig. 16. Correlation between seed set and grain size in the E plot 1956. Only the tetraploid plants are taken into consideration.

weight in a tetraploid population. Weak plants, aneuploids and others, as a rule have small grains as well as poor seed setting. This complicates the picture and there is not a straight line relation between seed setting and weight of grain in a tetraploid population.

Besides the plants mentioned which were sampled from each of the mixed populations, 20 samples equal to those taken in the vicinism experiments have been taken from the central part of each of the populations studied. This has given the average fertility of the plot, determined per ear and not per plant. The results are given in Table 14 and

TABLE 14. *Average seed set in samples of ears taken in the plots of mixed rye populations.*

	4 %	16 %	32 %	40 %	50 %	Pure stand 2x	Pure stand 4x
1954	53.9	52.6	—	—	41.4	—	60.9
1955	59.5	—	39.7	38.0	37.9	—	68.3
1956	64.1	50.2	45.1	—	46.9	81.1	70.0

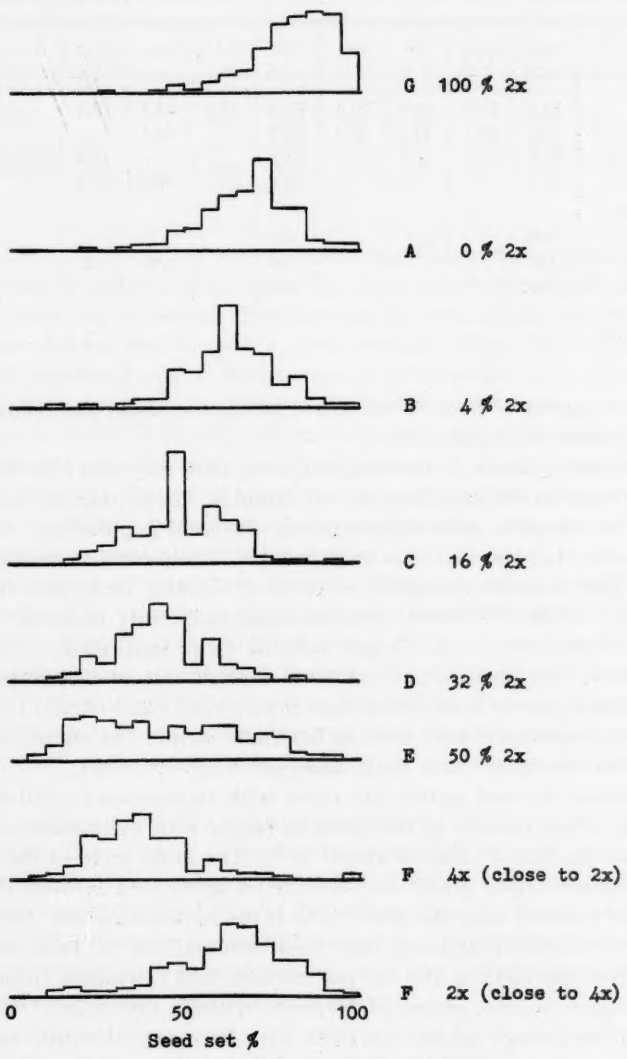


Fig. 17. Seed set in samples of ears from the lots of mixed rye populations 1956.

TABLE 15. *Yield of grain (dt/ha) from mixed populations.*

	0 %	0.5 %	1 %	2 %	4 %	8 %	16 %	32 %	40 %	50 %
1953	33.2	37.4	38.5	31.1	37.3	25.8	33.7	33.7	—	—
1954	28.5	28.1	24.5[1]	28.5	32.9	—	26.1	—	—	23.0
1955	23.0	—	—	—	21.5	—	—	16.3	18.2	16.8
1956	31.8	—	—	—	30.8	—	20.8	15.6	—	15.9
Average										
4 years	100				105					
3 »	100				108		86	70		
3 »	100				102					67

[1] lodging.

have been partly utilized in the above discussion about the relation between fertility and grain size.

A detailed analysis of the material from 1956 may serve to illustrate the methods used in handling the ear samples. The histogram in Fig. 17 shows the variation between ears in the different populations. An analysis shows that the variation in plot E (50 % diploids) is greater than that in plot A (pure tetraploid, $v^2=2.62$, $P<0.001$) or in plot G (pure diploid, $v^2=3.31$, $P<0.001$). The decrease in fertility from the A plot with 0 % diploids to the D plot with 32 % is significant ($v^2=87.76$, $P<0.001$). The even mixture of 50 % diploid and 50 % tetraploid in plot E had a poorer seed setting than the average value of plot F, where the two components were sown in alternate strips. The significance of this difference is not very high, however, $0.05>P>0.01$.

As shown the seed setting decreases with an increased admixture of diploids, while the size of the grain increases with the admixture until the latter reaches a value of about 16 %. The grain yield of the mixed plots depends upon grain size as well as upon seed setting. From a practical point of view the grain yield is the most important. The grain yields in the different years have been summarized in Table 15. The table gives the yield in 100 kg. per hectare, and calculated from plots, of 400 square metres, placed at distances of about 100 metres from each other. Even though all the rye plots have been placed within one and the same large wheat field it is evident that differences in soil productivity must have existed between the different plots. Close to each rye plot a wheat plot has been harvested in order to make a relative comparison possible. The results from 1956 are given in Table 16. From the

TABLE 16. *Yield of rye mixture and comparable wheat plots in 1956.*

Plot Frequency of 2x	A 0 %	B 4 %	C 16 %	D 32 %	E 50 %	F_{2x} 100 %	F_{4x} 0 %	G 100 %
Rye pop.	31.8	30.8	20.8	15.6	15.9	27.0	16.4	33.8
Wheat standard	25.3	23.0	25.2	29.3	35.2	30.8	30.8	33.7
Relative value rye/wheat	126	134	83	53	45	88	53	100

two tables 15 and 16 it is evident that a small admixture of diploids has not adversely influenced the yield. Up to an admixture of 4 % the yield has at least not decreased. The decrease in seed setting has been fully compensated by the increase in grain weight. When the frequency of diploids reaches 8—16 % the increase in grain weight is no longer sufficient to compensate the decrease in fertility and there is a marked decrease in yield. With still higher frequencies of diploids the average grain weight also decreases as already shown. The plot with 50 % diploids gave only about half the yield of pure tetraploid plots. If the frequency of diploids increases still further the yield must again increase towards the normal value for the diploid.

As already mentioned it is always difficult to compare yields from plots isolated by distance, and the indirect comparison using wheat as a standard suffers from the fact that wheat and rye do not react similarly to soil differences. According to Table 16 the tetraploid plot should have yielded not less than 26 % more than the diploid. The soil in the part of the field where the tetraploid plot grew may, however, be characterized as "rye-soil" while the diploid plot grew on more heavy soil, a typical "wheat soil".

Still another possibility to test the effect of diploids on the yield has been tried. The yield of straw has been weighed in each plot and the ratio of grain/straw has been calculated. It would be expected that this ratio was less influenced by soil differences than by the absolute yield of grain. The decrease in fertility caused by the admixture of diploids should be clearly expressed in this relation. The grain/straw ratio of the different mixtures in 1953 was as follows:

0 %	0.5 %	1 %	2 %	4 %	8 %	16 %	32 %
0.65	0.71	0.73	0.65	0.63	0.57	0.59	0.59

The differences are surprisingly small, but it may be noted that even in this comparison the highest values are obtained at a low admixture

of diploids. In spite of the difficulties encountered in judging the relative yields of different mixtures it seems fairly safe to conclude that a low admixture of diploids — 1—5 % — does not adversely influence the yield to such an extent that it has any economical importance.

VI. SEED CONTROL RULES AND OTHER PRACTICAL RECOMMENDATIONS

The sterility barrier which exists between tetraploid and diploid rye and which is mainly due to an abortion of triploid embryos is a good protection for artificial autotetraploid populations of rye. From the point of view of seed control the risk of contamination by spontaneous crosses with diploid may be considered as negligible. The very few triploid plants which will develop have such a low degree of fertility that they cannot genetically damage a tetraploid population. From this point of view diploid and tetraploid rye may be considered as two well separated species.

A distinctly different question is the influence upon the yield of a field of tetraploid rye of a neighbouring diploid field. Crosses between the two may cause a considerable loss of yield in the part of the tetraploid field closest to the diploid neighbour. According to the results here reported a distance of 100 metres is sufficient in most cases. The prevailing winds during flowering time are of considerable importance and *e.g.* in regions where these are mainly southwesterly it should be avoided to place a tetraploid field to the northeast of a diploid one of any considerable size.

In seed certification there must be a maximum limit to the admixture of diploids permitted. As has been shown the tetraploid population is self cleaning if the frequency of diploids is kept within reasonable limits; the critical value is 32 % or somewhat higher. From a genealogical point of view it would not be dangerous to permit 20 % diploids. On the other hand it must be taken into consideration that the buyer wants a maximum yield. To ascertain this a limit of about 4 % would be sufficient. Since it is rather easy to keep a tetraploid rye free from diploids the permitted admixture of these has in Sweden been fixed at 1 %, but from a biological point of view there does not seem to be anything against an increase of this value as far as to 4 %.

In rye the situation is very favourable because of the high critical value. For comparison it may be mentioned that the corresponding critical value for a mixture of diploids and tetraploids in red and alsike

clover is 3—4 % diploids (JULÉN and HAGBERG 1954). If there is a little more than 4 % of diploids in a tetraploid clover population the frequency of diploids will automatically increase in an accelerated tempo until the population is purely diploid. To preserve the tetraploid population it is necessary to keep the diploids below the critical value. Here the possibility to eliminate the small seeds of the diploid by sieving is a very good aid in keeping down the diploid frequency below the critical value.

As a result of the frequently irregular meiosis of the tetraploid there is a fairly high frequency of aneuploid plants, in Dubbelstål about 15 %. Most probably they cause a decrease in the yield of the population, but so far it has not been possible to analyze in detail their influence upon yield.

There is no doubt that on an average aneuploids are weaker than comparable euploids. Their fertility is low, and in the material here reported their seed setting is about one half of that of normal tetraploids. A considerable part of their progeny is probably also aneuploid (*cf.* MÜNTZING's result in *Dactylis*, 1937). It has been shown that hypotetraploids have smaller grains than the euploids and as pointed out earlier it is possible to reduce the frequency of hypotetraploids, by more than one half, by eliminating the small grained fraction of the tetraploid seed. At the same time possible admixtures of diploids will be mainly eliminated, but not the hypertetraploids. On the other hand it has been shown that low fertility is to some extent correlated with large grains and the use of only large-grained seed may result in decrease in fertility, more or less in combination with an increase of aneuploids. As shown in Fig. 16 the correlation between fertility and seed size is not represented by a straight line and many weak plants have both low fertility and small seeds. The elimination by sieving of a small-grained fraction of 20—30 % will probably not damage the population but have a beneficial influence on its composition and yield. Whether the increase in yield is sufficient to pay the increase in seed cost, resulting from the sieving, is at present the subject of further studies.

VII. SOME ASPECTS ON BREEDING TETRAPLOID RYE AND AUTOTETRAPLOIDS IN GENERAL

The importance of autopolyploidy in evolution has been discussed by several authors (*e.g.* MÜNTZING, 1936, CLAUSEN, KECK and HIESEY, 1945, STEBBINS, 1948, 1950, 1956, HAGBERG, 1953, *etc.*). The different points

of view on the problem has resulted in different opinions mainly due to the fact that the border line between auto- and allopolyploidy is not always quite sharp. Doubling the chromosomes of a fully homozygous self fertilizer gives the "strict" autopolyploid. When the chromosome number is doubled in a cross bred population containing different caryotypes — variation in chromosome structure, deficiencies, duplications, *etc.*, then this constitutes a step towards allopolyploidy.

A strict autopolyploid is not likely to be more successful than its original diploid. Generally the mere doubling of the chromosome number and the correlated increased cell size is a disadvantage. This has to be compensated by the new, rich possibilities of combinations and mode of inheritance of genes in the autopolyploids. A great genetic variation in a cross bred autopolyploid population is certainly much more promising as raw material for further breeding work. Such a population might be successful in nature; compare some cases of possible autopolyploidy: *e.g. Dactylis glomerata, Hordeum bulbosum, Phleum pratense, Medicago sativa etc.*

In the case of rye an autotetraploid population has not been established in nature without the plant breeders protection of the first tetraploid plants. The natural population would probably never reach the required frequency of 60 per cent tetraploids to prevent them from being eliminated rather quickly. However, as soon as the first autotetraploid population is established this population has a good competitive ability and is self cleaning with regard to competing diploids. The frequency of diploids has to be as high as about 40 per cent before they will become dangerous to the existence of the tetraploid population. The barrier of sterility between diploid and tetraploid rye is of significant importance as is described in the preceding chapters. It is not a question of an incompatibility barrier which is gametic as is common between two different but related species, but a question of zygotic lethality resulting in a decreased fertility and yield of seed. Genetically there is no exchange of hereditary material between a diploid and a competing tetraploid population. As indicated earlier diploid and tetraploid rye may in this respect be considered as two separate species (*cf.* CLAUSEN, KECK and HIESEY, 1945, pp. 150—151).

There are many other reasons for considering artificially produced autopolyploids as new distinct types separated from diploids. It has been shown that tetraploids often require other conditions for optimal development other than those required by the diploids. ELLERSTRÖM (1959 a and b) studied different levels of ploidy in *Phleum pratense* in field

trials all over the Scandinavian peninsula. The high ploidy levels gave the best results in areas with high rainfall and/or high latitude. This fact suggests that the testing of tetraploid populations under different environments other than that normal for the corresponding diploids is advisable. RASMUSSON (lecture for the polyploidy section of the Eucarpia, 1958) has given a very good illustration of this fact in tetraploid sugar beets. The experiences from testing a tetraploid Swedish spring rye population in California earlier mentioned is another example. The breeder has to find out which mode of cultivation is optimal for the tetraploids; density of stand, amount of fertilizer added and the different soils and climatic conditions in which the tetraploids are most productive. Day length reaction is another important factor to take into consideration in this respect. Thus, there is a good reason, for the exchange of autotetraploid populations between breeders in different regions, and testing them under widely different conditions. It is also important to bring as much genetic variation as possible into one and the same tetraploid population to create possibilities for complex gene combinations not possible at the diploid level. Selection of heterozygotes of the constitution $A_1A_2A_3A_4$ in such a "melting pot" will be a possibility to avoid homozygosity in the population and to create varieties with a high degree of heterozygosity. Thus, in breeding autotetraploid crops heterosis can be efficiently used where this is not conveniently possible in the corresponding diploids (*cf.* HAGBERG, 1953 c and 1955).

There are many other problems to be discussed in connection with the breeding of autotetraploids. A few might be briefly mentioned. Due to the mode of inheritance selection is a slower process in autotetraploids than in diploids. In the breeding of diploid cross fertilizers, "synthetics" and/or "F_1-varieties" are produced and released. On the tetraploid level the F_2 generation might have the maximum of heterozygosity. This is dependant on the genetic situation in the inbreds crossed and has to be tested in each case. Cross fertilization being one of the premises for a fairly rapid progress in "polyploidy breeding" made possible by heterozygosity, the self incompatibility system and its effect and function on the polyploid level is very important as is pointed out by several authors, *e.g.* LEWIS, 1954 and 1956, ATWOOD and BREWBAKER, 1953, and LUNDQVIST, 1957. According to LUNDQVIST the system in rye is functioning better in populations with large genetic variation while in clover the opposite phenomenon seems to occur. In the breeding programme for the tetraploids this has to be taken into consideration.

As to the pairing conditions at meiosis MÜNTZING (1951) found no differences in rye after 7 generations of intense selection for high fertility. Similar results were obtained by MORRISON (1956), while GILLES and RANDOLPH (1951) in maize found a slight reduction of quadrivalent frequency during a period of 10 generations. In rye HILPERT (1957) also found a slight effect of selection for regular distribution of chromosomes at meiosis. The present paper, however, demonstrates, that an increase in fertility is correlated with decrease in seed size and the yield will be the same. If the fertility is decreased as in the plots of 1—4 per cent diploid admixtures the seed size is increased correspondingly and the yield seems to be the same. To increase yield it is necessary to select types which have a higher assimilation capacity and a more "economical" distribution and use of the assimilates giving a higher carbohydrate production per unit area of field. Studies of this kind are now in progress.

A question, which is often discussed, concerns the constancy of the autotetraploid populations. There are cases described where tetraploids show diploid tissues in premeiotic stages and thus diploids in the progeny (*e.g.* GOTTSCHALK, 1958). In a cross fertilizing population such as the autotetraploid rye there is very little chance that diploids eventually formed could in one generation reach the frequency of 30—40 per cent of the individuals in the population required for the diploids to become dominant. Below this frequency of diploids the tetraploid rye is "self cleaning", since single diploids will be sterile. Thus, there is practically no risk of this phenomenon causing the loss of an autotetraploid population of rye.

In the strict autotetraploid populations of the self fertilizers, flax and barley diploids some times occur. These diploids will rapidly increase in the population because of their higher fertility (HAGBERG and ELLERSTRÖM, in manus). It was not possible to decide whether these plants were admixtures or were spontaneously arising. Since 1951 the chromosome number in every plant has been checked in a population of flax. In this very effectively controlled population of flax, diploid plants have never been obtained. However, if diploids are formed in tetraploid populations of self fertilizers there is a great risk of a rapid increase of diploids among the tetraploids.

It is true that autotetraploid populations of rye probably could never arise in nature. However, it is also true, that tetraploid rye plants compete very well with diploids at least under Swedish conditions, and that spontaneously arisen diploid plants would never be able to turn a tetra-

ploid rye population back to diploidy. With the breeders aid tetraploid rye populations can be established and no doubt they constitute a useful and valuable raw material for promising, future breeding work.

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SUMMARY

The present paper presents and discusses a collection of relevant data from some investigations on tetraploid rye carried out at the Swedish Seed Association during the last 10 years. The main aim of these investigations was to forestall certain practical difficulties which might be encountered in trying to establish tetraploid rye as a worthwile crop in practical farming.

The frequency of aneuploids has been determined for 9 different seed lots belonging to the tetraploid variety "Dubbelstål". As an average 14.7 % of the seeds are aneuploids, but the frequency was found to vary considerably between different seed lots, the extreme values being 6.8 % and 20.4 %.

The reduction in the seed yield of tetraploid rye caused by the presence of aneuploids is discussed. Figures are given for the reduction of the frequency of aneuploids especially hypoploids, brought about when the small-grained portion of a seed lot is removed.

The seed setting is reduced in tetraploid rye when it is exposed to pollen from diploid rye. This is due to the fact that, in the style of the tetraploid rye, the pollen tube, derived from a haploid pollen grain, grows faster than a pollen tube derived from a diploid pollen grain. Triploid embryos are formed, which show irregular development and, for the most part, disintegrate at an early stage. When diploid plants are heavily exposed to the pollen of tetraploids, the seed setting of the diploids is also reduced due to the abortion of the triploid embryos formed.

The seed setting and yield have been studied in fields of tetraploid rye exposed to pollen from fields of diploid rye. In a small field of tetraploid rye, totally surrounded by diploid rye, the reduction in yield was estimated to be about 30 %. However, fields of tetraploid rye grown in areas, where much diploid rye is grown yielded a normal crop. The

haploid "pollen cloud", which must dominate such an area, seems to be of minor importance in this case.

By studying the seed fertility of tetraploid rye grown in plots at various distances from a large field of diploid rye, it was found that a distance of 100 metres, between a diploid and a tetraploid field, is sufficient to avoid the deleterious effect of intercrossing. However, it is pointed out that the minimum distance is influenced by the prevailing winds during the flowering period.

In applying tetraploid rye to practical farming there is always a certain risk of the mechanical admixture of diploids in the tetraploid populations. The effect of such an admixture has been investigated in tetraploid rye to which diploid rye was admixed in frequencies ranging from ½ % to 50 % of the total population. A threshold value was found to exist at a frequency of diploids lying somewhere between 32 % and 40 %. At this threshold value the two levels of ploidy are maintaining the original frequency in the progeny. The tetraploid is "self-cleaning" when the frequency of diploids admixed is lower than 32 %. The seed yield of the mixed populations is not reduced, in relation to that of pure tetraploid populations, until the frequency of diploids is higher than 4 %. At this frequency the decrease in seed setting is compensated by a corresponding increase in 1000-grain weight.

In seed certification there must be a minimum limit to the admixture of diploids permissable in tetraploid rye. Since it is rather easy to keep a tetraploid rye free from diploids this limit has already been set at 1 %.

The possibility of improving the yield of tetraploid rye, by sifting off the small sized portion of the seed lot, is discussed. In this way the frequency of aneuploids will be reduced. On the other hand it has been shown that low fertility is to some extent correlated with large grain size. Thus, too severe a sifting may reduce the yield, and give a higher frequency of aneuploids. However, since the relationship between fertility and seed size is not a straight line graph, the removal of a small grained portion, equivalent to 20—30 % of the seed lot, will probably have a beneficial influence on the yield.

Some problems concerning the breeding of autopolyploids are discussed. The reasons for considering artificially produced autopolyploids as new distinct types are pointed out. The fact, that polyploids very often have quite different requirements, compared to those demanded by the corresponding diploids, for optimal growth, makes it advisable for breeders in different regions of the world to exchange polyploid material for testing under widely different conditions.

The importance of bringing as much genetic variation as possible into one and the same tetraploid population, in order to create possibilities for new complex gene combinations, is pointed out. In the breeding of autotetraploid crops heterosis can be efficiently used where this is not conveniently possible in the corresponding diploids.

Since cross fertilization is one of the premises for progress in "polyploidy breeding", the self incompatibility system of the plant has to be taken into consideration. The system in rye functions better, in tetraploid populations, the larger the genetic variation of the tetraploid population is, whilst in tetraploid clover, for example, the situation is reversed.

Selection for a regular distribution of chromosomes at meiosis, resulting in higher seed fertility, has had only a slight effect if any. It is also demonstrated in the present paper that an increase in fertility is correlated with a decrease in seed size, resulting in the same yield of grain. To improve yield it is therefore necessary to select for other characters, such as high assimilation capacity, and a more economical distribution and utilisation of the assimilates within the plant.

In some cases tetraploid populations have been reported to give rise to diploid individuals, through some kind of chromosome reduction in premeiotic stages. In all seed lots of tetraploid rye, so far studied, no diploids have been found. The "self-cleaning" capacity of tetraploid rye serves as a strong protection against the possibility of diploids, eventually formed, reaching the frequency of 30—40 % necessary to enable the diploids to become dominant in the population. In this respect tetraploid rye differs strongly from autotetraploids of self fertilizing species, where there is a great risk of a rapid increase of diploids, because of their higher fertility. Further, tetraploid rye also differs from crops, such as sugar beets, where triploids are developed into mature plants.

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A TRANSLOCATION TESTER SET IN PISUM

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I. INTRODUCTION

THE species *Pisum sativum* is not only a classical object of genetic investigation but also a cultivated plant of long and continued interest to the breeder. Hence it is astonishing that a detailed and indisputably correct linkage map involving the entire chromosome set and the position of the centromeres has not yet been constructed. In order to facilitate the mapping work a translocation tester set involving each of the seven chromosomes has been collected and is described here.

With regard to the mapping work the use of translocations is more advantageous than the sole use of genes since semisterility is a better marker than at least some of the genes in *Pisum*, and the reduction of crossing-over which often occurs in the neighbourhood of the points of interchange increases the length of chromosome over which the test is effective. In addition, two chromosomes are tested instead of only one.

An ideal tester set should have one point of interchange in the median segment of each chromosome arm and should also contain a number of well distributed suitable marker genes. At present such a set is not available in *Pisum*. The points of interchange in the tester set presented here are predominantly proximal to the centromeres and the chromosomes are unsatisfactorily tagged with marker genes.

Despite the imperfection of this tester set its identification and use in mapping work have produced some new results concerning the genetic map of *Pisum* as well as suggestions about the probable location of several of the centromeres on this map. Up to the present four of the seven chromosomes have been related to linkage groups by these cytogenetical studies, while the three remaining chromosomes have not yet been tagged with suitable marker genes.

II. NOMENCLATURE AND METHODS

In cytogenetically well studied species, *e.g. Zea mays* and *Lycopersicon esculentum*, a standardized generally accepted nomenclature has

been established, but such is not the case in *Pisum*. In the present paper the chromosomes were given Arabic numerals according to SANSOME (1937 and 1938). Translocations were symbolized by a *"T"*; and the chromosomes involved were indicated by their respective numbers, which were separated by a hyphen and placed in parenthesis following the *"T"*. In order to distinguish between translocations involving the same chromosomes, lowercase letters are used following the last parenthesis (*cf.* BARTON *et al.*, 1955). The marker gene symbols commonly employed in *Pisum* genetics were used.

In the F_1 hybrids pollen fertility was determined from counts made on pollen which had been dispersed and mounted on slides in equal parts of aceto-carmine and glycerine. No unequal distribution of normal and shrivelled pollen grains was noted on any of the slides.

The discrimination between fertile and semisterile plants in F_2 progenies was made in the field by microscopic inspection of dry pollen at low magnification ($\times$ 50). At the same time trisomic plants, which appear in low frequency, were marked. These plants usually deviate morphologically from the diploids (*cf.* NILSSON, 1936; PELLEW, 1940) and are more fertile than their semisterile siblings (SUTTON, 1939). The seed set of F_2 individuals may also be used to distinguish between fertility and semisterility, but this method is sometimes less reliable than pollen inspection. Thus adverse weather condition may reduce the seed setting of weak genotypes. In certain genotypes *e.g.* those homozygous for *cri* the pollen fertility is normal whereas the seed setting is reduced because of the crisp or folded structure of the carpels (LAMM, 1949).

The estimation of linkage was made according to the maximum likelihood method using the procedures proposed by FINNEY (1949) and elaborated by LAMM (1950). In cases with deviating single factor ratios, however, the product method has been used (*cf.* JOACHIM, 1947). Pooled or combined estimates have been restricted to cases without significant heterogeneity. Statistical significance has been marked with one, two or three asterisks corresponding to the conventional levels of *P*.

The pairing behaviour of the chromosomes at first division metaphase of meiosis in the various translocation heterozygotes was studied in squash preparations of PMC's. Most of the plant material was grown in cool greenhouses during the autumn and winter. The plants grown in the winter were given supplementary artificial light from fluorescent lamps.

The cytological techniques employed were essentially those of LAMM (1951) and LIMA-DE-FARIA (1948 and 1952). The fixation of buds col-

lected from field-grown plants in the hot days of July gave PMC's with darkly staining cytoplasm, which was inferior to that of buds collected from greenhouse-grown plants. Iron-aceto-carmine stain of about 1 % carmine concentration was used. In separating the cover slip from the slide preparatory to making the squash permanent the CO_2-freezing technique described by CHRISTIANSEN (1957, p. 28) was tried with good success. Much fewer cells were lost by this method than by the older acetic acid method of separation.

III. CYTOLOGICAL STUDIES

The "basic" tester set

The tester set of the present paper contains eight different spontaneous reciprocal translocations. Their heterozygotes with the normal or standard structural type give a maximum association of one ring-of-four and five bivalents at MI of meiosis. Of these translocations five have been selected as suitable for a "basic" tester set, since each of the seven chromosomes is involved and the ring formation is comparatively regular due to a more or less proximal localization of the points of interchange. Studies on the remaining three translocations will be presented in the next section.

A survey of meiotic investigations on F_1 hybrids from diallel crosses within the basic tester set and from crosses with the normal structural type is given in Table 1. The maximum ring and bivalent formation is shown by the usual symbols, and available data on the pollen sterility are given.

References and descriptions concerning the meiotic behaviour of *T(1—2)a* and *T(1—3)b* have been given by LAMM (1951). In that paper they were presented as prime type B and D, prime type A being the normal structural type. In the hybrid *T(1—3)b*×normal (combination No. 2 of Table 1) a chiasma is occasionally formed in one of the two interstitial segments (*cf.* LAMM, 1951, Figs. 4—5).

T(3—4)a originally appeared in descendants of a cross between Duke of Albany and a non-ring-forming Thibetan variety (PELLEW and SANSOME, 1934). It was called Merton 1 and designated as structural type 8 (interchange between chromosomes 3 and 4) by SANSOME (1938). In hybrids with the normal structural type (combination No. 3 of Table 1) a fairly regular ring-of-four is formed (see Fig. 1); and this ring is also easily recognized in hybrids with other structural types (*cf.* Combinations 8, 12, 13 of Table 1 and Figs. 4, 5, 6).

TABLE 1. *Survey of the results of meiotic investigations in the "basic" tester set.*

Translocation name source line	The normal structural type	*T (6—7) a* N II L. 108	*T (5—6) a* N I L. 114	*T (3—4) a* Merton 1 L. 111	*T (1—3) b* E. Rapid L. 83
T (1—2) a K-line HAMMARLUND L. 84	No. 1 ⊙ 4 + 5 II 44 % H., 1931, Figs. 19—22	No. 6 2 ⊙ 4 + 3 II 77 % Fig. 2	No. 10 2 ⊙ 4 + 3 II H., unpubl.	No. 13 2 ⊙ 4 + 3 II Fig. 6	No. 15 ⊙ 6 + 4 II 68 % L., 1951, Figs. 14—18
T (1—3) b Extra Rapid N NILSSON L. 83	No. 2 ⊙ 4 + 5 II 51 % L., 1951, Figs. 1—7	No. 7 2 ⊙ 4 + 3 II Fig. 3	No. 11 2 ⊙ 4 + 3 II H., 1934, Fig. 3	No. 14 ⊙ 6 + 4 II 59 % Fig. 7	
T (3—4) a Merton 1 PELLEW L. 111	No. 3 ⊙ 4 + 5 II Fig. 1	No. 8 2 ⊙ 4 + 3 II 66 % Fig. 4	No. 12 2 ⊙ 4 + 3 II Fig. 5		
T (5—6) a N I NILSSON L. 114	No. 4 ⊙ 4 + 5 II 50 % H., 1932, Fig. 1	No. 9 ⊙ 6 + 4 II H., 1934, Fig. 2			
T (6—7) a N II NILSSON L. 108	No. 5 ⊙ 4 + 5 II 50 % H., 1932, Fig. 2				

Key to the squares of the survey.

Combination number
Max.ring and bivalent formation
% sterile pollen
Literature and Figs.,
H. = HÅKANSSON
L. = LAMM

T(5—6)a and *T(6—7)a* were found by NILSSON (see NI and NII in NILSSON's paper of 1936). They were cytologically studied by HÅKANSSON (1932) and are presented as structural types 11 and 10 by SANSOME (1938). It seems likely from the present investigation where the corresponding rings have been recognized in various hybrids that the chromosomes involved in *T(6—7)a* are comparatively small (see Figs. 2, 3, 4, 11 and 13).

The hybrids in the survey of Table 1 completely agree in their pairing behaviour with the expectations based on the chromosomes as-

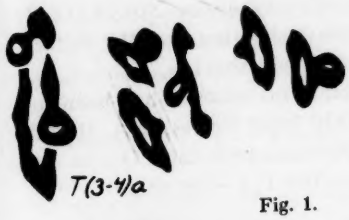


Fig. 1.

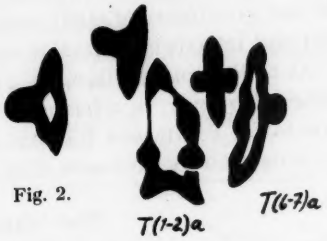
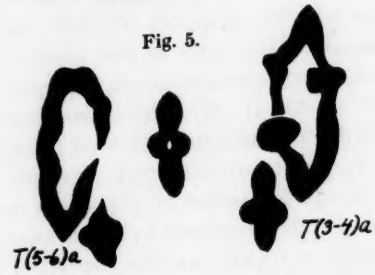


Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.



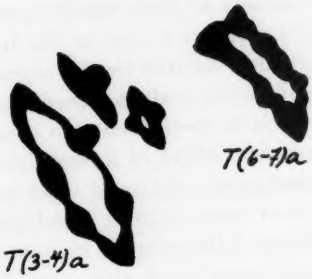


Fig. 7.

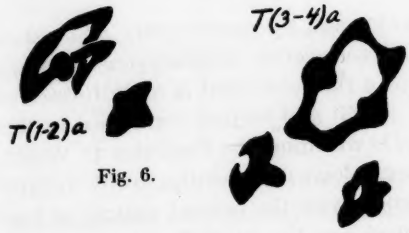


Fig. 6.

Figs. 1—7. MI of meiosis in the PMC's of some translocation heterozygotes belonging to the "basic" tester set. Fig. 1. *T(3—4)a*×normal. — Fig. 2. *T(6—7)a*×*T(1—2)a*. — Fig. 3. *T(6—7)a*×*T(1—3)b*. — Fig. 4. *T(6—7)a*×*T(3—4)a*. — Fig. 5. *T(5—6)a*× ×*T(3—4)a*. — Fig. 6. *T(1—2)a*×*T(3—4)a*. — Fig. 7. *T(1—3)b*×*T(3—4)a*. — (×2500).

sumed to be involved. The rings-of-six (Combinations Nos. 9, 14, 15) do not give figure-of-eight configurations, indicating that the differential and interstitial segments are comparatively short.

As to the combination No. 10 of Table 1 no account has been published. However, in a letter of March 1940 from Professor A. HÅKANSSON to Dr. E. NILSSON it is stated that there are two rings. One of these is recognized by Professor HÅKANSSON as the *T(1—2)a* ring.

The "extended" tester set

The "extended" tester set is here defined to include the "basic" tester set plus three other translocations whose chromosomal constitutions have been verified through crosses with appropriate members of the entire set. These translocations *i.e.* *T(1—3)a*, *T(1—4)a* and *T(3—5)a* are listed in the first row of squares of Table 2.

Common to these three translocations is a comparatively long interstitial segment in one of the translocated chromosomes. A chiasma is frequently formed in this segment, with the effect that the connected crossing-over values are reduced (LAMM, 1948; BURNHAM, 1956 a), which is of special value in connection with the mapping of genes. The cytological effect of interstitial chiasmata is the more or less frequent formation of a figure-of-eight involving four chromosomes in the translocation heterozygotes (see Figs. 8, 9, 12, 13, 21, 22). The more distal position of the point of interchange in one chromosome involved in the translocation may give comparatively short interchanged segments, which results in decreased chances for the formation of chiasmata in these segments. Hence the ring-of-four is often replaced by other associations, *e.g.*, two bivalents, open chains or frying-pan-like configurations.

T(1—3)a was described by PELLEW and SANSOME (1931), who called this structural type Thibet 7. In translocation heterozygotes between this and the normal structural type a figure-of-eight is often formed at meiosis as observed by SANSOME (1933) and further confirmed in this study (see Figs. 21, 22, 25). *T(1—4)a* was found by Professor Ø. WINGE and named after him. It has been cytologically investigated by SUTTON (1935). In translocation heterozygotes with the normal structural type a figure-of-eight is frequently formed (see Fig. 8). *T(3—5)a* was found in 1929 by Dr. E. NILSSON (see NILSSON, 1950). The translocation heterozygote with the normal structural type was called the NIII case by Professor A. HÅKANSSON, who studied the pairing at meiosis (HÅKANSSON, 1932).

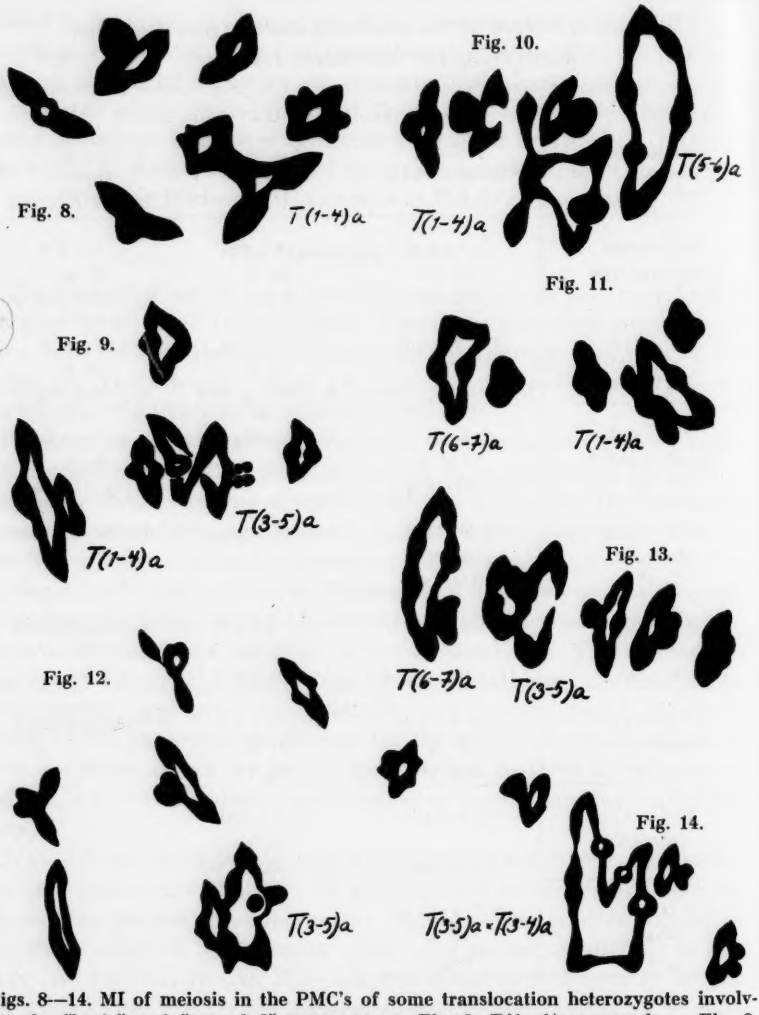
TABLE 2. *Survey of the results of meiotic investigations concerning the "extended" tester set.*

Translocation Name Line	The "extended" tester set.		
	T (3—5) a NILSSON'S N III L. 88	*T (1—4) a* WINGE L. 112	*T (1—3) a* Thibet 7 L. 87
The normal structural type	⊙ 4 + 5 II 50 % Fig. 12	⊙ 4 + 5 II 33 % Fig. 8	⊙ 4 + 5 II 47 % SA., 1933. Fig. 4
T (1—2) a K-line L. 84	2 ⊙ 4 + 3 II 70 % L., 1951, Figs. 19-22	⊙ 6 + 4 II 75 % SU., 1935, p. 690	⊙ 6 + 4 II 70 % SA., 1932, Fig. 2
T (1—3) b Extra Rapid N L. 83	⊙ 6 + 4 II > 50 % H., 1934, Fig. 1	Not investigated	"4" + 5 II 65 % Fig. 15—18
T (3—4) a Merton 1 L. 111	⊙ 6 + 4 II 61 % Fig. 14	Not investigated	Not investigated
T (5—6) a NILSSON'S N I L. 114	⊙ 6 + 4 II > 50 % H., 1934, Fig. 4	2 ⊙ 4 + 3 II 78 % Fig. 10	Not investigated
T (6—7) a NILSSON'S N II L. 108	2 ⊙ 4 + 3 II 67 % Fig. 13	2 ⊙ 4 + 3 II 77 % Fig. 11	Not investigated
T (1—3) a Thibet 7 L. 87	⊙ 6 + 4 II H., unpubl.	⊙ 6 + 4 II 75 % SU., 1935, p. 690	
T (1—4) a WINGE L. 112	2 ⊙ 4 + 3 II 71 % Fig. 9		

Key, cf. Table 1.

H. = HÅKANSSON; L. = LAMM; SA. = SANSOME; SU. = SUTTON.

The chromosomal constitution of Thibet 7, Winge and NIII has been thought to be *T(1—3)a*, *T(1—4)a* and *T(3—5)a*, respectively. Although the cytological evidence for the verification of the assumed chromosomal constitution is yet not based on complete diallel crossings the



Figs. 8—14. MI of meiosis in the PMC's of some translocation heterozygotes involving the "basic" and "extended" tester set. — Fig. 8. *T(1—4)a*×normal. — Fig. 9. *T(3—5)a*×*T(1—4)a*. — Fig. 10. *T(1—4)a*×*T(5—6)a*. — Fig. 11. *T(1—4)a*×*T(6—7)a*. — Fig. 12. *T(3—5)a*×*normal*. — Fig. 13. *T(3—5)a*×*T(6—7)a*. — Fig. 14. *T(3—5)a*×*T(3—4)a*. — (×2500).

results given in Table 2 are hitherto in agreement with these assumptions. Figures 10—14 illustrates the new contributions of this paper to the cytological analysis given in Table 2.

TABLE 3 a. *Crosses investigated.*

Cross No.	Parental lines ♀	♂	Percent germ.	Nos of plants	Translocation introduced by one of the parental lines	Genes of translocated line
1	84	135	81	245	HAMMARLUND'S K=*T(1—2)a*	*A Le Tl*
2	84	7	85	342	„ „ „	*A* Vi_1
3	see text		—	91	Early Giant Rogue=*T(1—2)b*	*a*
4	„	„	—	99	„ „ „ „	„
5	„	„	85	46	Thibet 7=*T(1—3)a*	*Gp*
6	87	44	70	192	„ „ „	*A Gp*
7	87	7	97	161	„ „ „	*R Tl Le*
8	87	7	98	321	„ „ „	„ „ „
9	87	6	85	137	„ „ „	*I Wa Le*
10	87	6	93	149	„ „ „	„ „ „
11	31	87	87	278	„ „ „	*St B Le*
12	153	176	84	65	From Thibet 7=*T(1—3)a*	*R I le*
13	154	176	96	221	„ „ „ „	„ „ „
14	155	176	94	134	„ „ „ „	„ „ „
15	83	67	83	205	Extra Rapid N=*T(1—3)b*	*a F* u_{st}
16	83	26	80	261	„ „ „ „	*a b r i Le m*
17	40a	83	86	192	„ „ „ „	*Wa*
18	23	83	90	101	„ „ „ „	*Was*
19	112	44	94	276	WINGE=*T(1—4)a*	*A Gp*
20	44	112	89	276	„ „	„ „
21	112	24	96	167	„ „	*R I Le N Wa*
22	119	111	88	86	Merton 1=*T(3—4)a*	*St B Le*
23	24	111	82	152	„ „ „	*Le Wa*
24	23	111	91	200	„ „ „	*I R Wa*
25	90	88	93	293	NILSSON'S NIII=*T(3—5)a*	*Le Lat*
26	88	30	78	211	„ „ „	*Le St Wlo*
27	114	6	94	141	NILSSON'S NI=*T(5—6)a*	*I Le Wa*
28	6	114	91	160	„ „ „	„ „ „
29	114	116	73	335	„ „ „	*Gp Le*
30	114	3	100	90	„ „ „	*Le Pl*
31	3	114	95	107	„ „ „	„ „
32	108	3	98	296	NILSSON'S NII=*T(6—7)a*	„ „
33	108	24	94	247	„ „ „	*Le Wa I R N*
34	108	24	93	93	„ „ „	„ „ „ „ „
35	24	108	78	87	„ „ „	„ „ „ „ „ „
36	30	108	83	148	„ „ „	*Wlo*
37	30	108	80	128	„ „ „	„

IV. GENETICAL STUDIES

In the previous chapter an account is given of the cytological determination of the chromosomes involved in the various translocations. It now remains necessary to associate these cytological chromosomes with

TABLE 3 b. *Statistical analysis of F_2 progenies from crosses given in Table 3 a.*

Cross No.	Factors* involved 1	2	Categories[1] and Nos. of individuals a	b	c	d	n	χ^2 linkage 1–2	Percent crossing over
1	*T(1—2)a*	*Le*	141	44	117	40	342	0.14	
1	„	*Tl**	153	32	121	36	342	1.89	
1—2	„	*A*	165	133	278	11	587	130.29***	4.2 ± 1.27
2	„	„	62	51	122	10	245	45.56***	7.7 ± 2.26
2	„	*Vi_1**	69	44	129	3	245	45.56***	3.2 ± 1.88
2	*A*	„	178	6	20	41	245	99.75***	11.8 ± 2.22
3—4	*T(1—2)b*	*A*	54	32	95	9	190	21.23***	10.8 ± 3.29
5 (BC)	*T(1—3)a*	*Gp*	5	17	19	5	46	14.70***	
6	„	„	56	38	85	13	192	18.78***	
5—6	„	„					(311)	32.34***	16.9 ± 3.28
6	„	*A*	75	19	70	28	192	1.78	
7—8	„	*R*	131	102	236	13	482	97.70***	5.7 ± 1.44
7—8	„	*Tl*	131	102	239	10	482	101.97***	4.5 ± 1.34
7—8	*R*	„	363	4	7	108	482	390.78***	2.4 ± 0.70
9—10	*T(1—3)a*	*I*	110	22	110	44	286	5.08*	no linkage
9—10	„	*Wa*	97	35	114	40	286	0.01	
11	„	*B*	65	69	134	10	278	72.56***	5.9 ± 1.99
11	„	*St*	96	38	113	31	278	1.73	31.5 ± 6.97
11	*B*	„	164	40	30	39	278	38.41***	28.3 ± 3.27
7—11	*T(1—3)a*	*Le*	383	116	417	130	1046	0.02	
12—14	„	*R*	106	88	204	22	420	65.54***	9.6 ± 1.93
12—14	„	*I*	145	49	169	57	420	0.00	
12—14	*R*	„	234	76	80	30	420	0.34	
15	*T(1—3)b**	*F*	38	20	76	16	150	5.56*	21.5 ± 5.82
15	„	*U_{st}*	31	27	82	10	150	23.12***	9.9 ± 3.57
15	*F*	„	79	35	34	2	150	8.96**	24.0 ± 7.61
16	*T(1—3)b**	*Le*	76	34	112	39	261	0.56	
16	„	*Cri*	61	49	138	13	261	43.71***	8.6 ± 2.48
16	„	*M*	54	21	88	28	191	0.30	
16	*U_{st}*	„	112	42	30	7	191	0.98	
15—16	*T(1—3)b*	*A*	143	45	217	61	466	0.48	
17	„	*Wa*	68	26	79	19	192	1.78	
18	„	*Was*	34	7	40	20	101	3.59	
19—20	*T(1—4)a*	*A*	186	62	206	60	514	0.44	
19—20	„	*Gp*	173	75	211	55	514	6.23*	28.3 ± 4.28
21	„	*R*	47	39	80	1	167	43.13***	1.4 ± 1.38
21	„	*I*	66	20	65	16	167	0.24	
21	„	*Le*	67	19	65	16	167	0.10	
21	„	*N*	65	21	62	19	167	0.02	
21	„	*Wa*	71	15	57	24	167	3.35	
22	*T(3—4)a*	*B***	18	26	36	6	86	23.58***	8.5 ± 4.32

TABLE 3 b. Continued.

Cross No.	Factors* involved 1	2	Categories[1] and Nos. of individuals a	b	c	d	n	χ^2 linkage 1–2	Percent crossing over
22	*T(3—4)a*	*St*	28	16	41	1	86	13.04***	4.1 ± 2.97
22	*B***	„	53	1	16	16	86	27.54***	10.8 ± 3.58
22—23	*T(3—4)a*	*Le*	83	24	94	37	238	1.10	
24	„	*I*	81	21	71	27	200	1.31	
24	„	*R*	65	37	75	23	200	4.51*	(26.0 ± 6.26)
23—24	„	*Wa*	124	41	136	51	352	0.31	
25	*T(3—5)a*	*Lat*	116	31	112	34	293	0.19	
26	„	*Wlo*	81	29	73	28	211	0.04	
26	„	*St*	83	27	79	22	211	0.19	
26	*Le*	„	129	32	33	17	211	3.99*	39.9 ± 4.56
25—26	*T(3—5)a*	*Le*	170	87	202	45	504	16.51***	22.4 ± 3.34
27—28	*T(5—6)a*	*I*	119	28	117	37	301	0.93	
27—28	„	*Wa*	111	36	106	48	301	1.86	
29	„	*Gp*	126	44	119	46	335	0.16	
31	„	*Pl*	42	10	45	10	107	0.03	
27—31	„	*Le*	313	105	329	86	833	2.13	
32	*T(6—7)a*	*Pl*	131	28	104	33	296	1.99	
32—35	„	*Le*	271	85	284	83	723	0.17	
33—35	„	*Wa*	138	59	178	52	427	2.91	
33—35	„	*I*	143	54	170	60	427	0.06	
33—35	„	*R*	153	44	161	69	427	3.50	
34—35	„	*N*	65	25	61	29	180	0.47	
36—37	„	*Wlo**	109	24	115	28	276	0.04	

[1] Categories

Factor 1 = Translocation (point of interchange)	Factor 1 = Marker Gene 1
„ 2 = Marker Gene	„ 2 = „ „ 2
„ a = Fertile — Dominant	„ a = Dominant 1 — Dominant 2
„ b = „ — Recessive	„ b = „ 1 — Recessive 2
„ c = Semisterile — Dominant	„ c = Recessive 1 — Dominant 2
„ d = „ — Recessive	„ d = „ 1 — Recessive 2

* Significant deviation in single factor ratios.

the genetic ones. This involves the detection and estimation of linkage between the marker genes of two genetic chromosomes and the point of interchange of a given translocation. The results of these linkage determinations are given in Table 3 a—3 b. On the basis of these and previous investigations the following conclusions regarding the separate chromosomes may be drawn.

Chromosome 1

Translocations involving chromosome 1 show in each case linkage between their point of interchange and the gene *Gp*. Further evidence that the chromosome containing *Gp* is involved in these translocations is to be found in the linkage between the point of interchange in *T(1—3)b* and the genes *Ust* and *Fs*. The Marker gene *R* is also often found to be linked with the point of interchange in these translocations. Thus this gene must, as the present result confirms, belong to the *Gp* chromosome and not to a different one (*cf.* LAMM, 1956). The linkage values found in progenies of translocation heterozygotes involving chromosome 1 are given below.

Translocations names	symbols	Marker genes	Linkage values	References
HAMMARLUND's K	*T(1—2)a*	*Gp*	about 1 %	PELLEW, 1940
„	„	*Fs*	„ 20 „	„ „
„	„	*R*	„ 40 „	„ „
Thibet 7	*T(1—3)a*	*Gp*	16.9 „	Table 3 b
„	„	*R*	5.5 „	LAMM, 1951
„	„	*R*	5.7 „	Table 3 b
„	„	*Tl*	4.5 „	„ „
from Thibet 7	„	*R*	9.6 „	„ „
Extra Rapid N	*T(1—3)b*	*Gp*	6.5 „	LAMM, 1949
„ „ „	„	*Fs*	12.2 „	„ 1948
„ „ „	„	*Ust*	23.1 „	Table 3 b
„ „ „	„	*R*	(46.2)[1] %	NILSSON, 1950
WINGE	*T(1—4)a*	*Gp*	28.3 %	Table 3 b
„	„	*R*	1.4 „	„ „

[1] The linkage value *T(1—3)b—R* is not significant and hence put in parenthesis.

In the translocation heterozygote between Thibet 7 and the normal structural type only chromosomes 1 and 3 are involved in the translocation. Hence genes situated in other chromosomes should give independent segregation with loci or other structures belonging to chromosomes 1 and 3. In progenies raised from the translocation heterozygote mentioned above this has not always been the case.

PELLEW (1937) claims that *A* is situated in the *R* chromosome *i.e.* chromosome 1 of the present investigation. The assumed linkage between *T(1—3)a* and *A* was, however, not supported by the data given by LAMM (1951) or by the new data given in Table 3 b.

A significant linkage between *Le* and *R* was shown by LAMM (1951, pp. 366—367). This linkage was supported by back-crosses to the translocation heterozygotes giving unexpected linkage relations of various strength between the gene *Le* of chromosome 5 and *T(1—3)a*, *Pa*, *R*, *Tl* and *Bt* of chromosome 1 (NILSSON, unpubl.). According to crosses Nos. 7—11 (see Table 3 b) of the present investigation there is no linkage between *T(1—3)a* and *Le*. In the progenies of the crosses Nos. 7—8 (see

Table 3 a) no linkage was found neither between *T(1—3)a* and *Le* nor between *Le* and *R* whereas the linkage between *T(1—3)a* and *R* amounted to 5.7 ± 1.44 %.

A confusing behaviour of the *I* gene in progenies of the Thibet 7 translocation heterozygote was found by PELLEW and SANSOME (1931). The data given by LAMM (1951) indicate a slight linkage between *T(1—3)a* and *I* and between *I* and *R*. Crosses Nos. 9—10 of Table 3 b, however, show a reverse tendency of the linkage first mentioned. As will be mentioned later *I* is probably situated in chromosome 2.

Perhaps the unexpected behaviour of *A*, *Le* and *I* discussed above is only accidental. There is, however, a slight possibility that a quasi-linkage due to so-called affinity (see WALLACE, 1958) may sometimes be operating. The heterozygote between Thibet 7 and the normal structural type is heterocentric for all chromosomes involved. A slight tendency for a non-random distribution of the centromeres of Thibet 7 and the normal structural type would explain the quasi-linkage sometimes observed.

By suitable crosses a *T(1—3)a* structural type has been produced which is heterocentric for chromosome 1 (the *R* chromosome) but homocentric for chromosome 2 (the *I* chromosome). In crosses Nos. 12—14 of Tables 3 a— 3 b this type was used as one of the parents. Table 3 b shows that no linkage between *T(1—3)a* and *I* nor between *R* and *I* could be detected in these crosses. Although this is by no means a proof of the probable occurrence of affinity in *Pisum* a study of this phenomenon in crosses between *Pisum* lines of widely differing geographical origin would perhaps be of some interest.

In this connection it should be mentioned that terminal knobs have been observed on some of the *Pisum* chromosomes at pachytene. These knobs could perhaps be the seat of new centric activities to which preferential segregation in maize has been attributed by RHOADES (1952). Once more, however, it should be pointed out that the occurrence of interaction across the cytoplasm between certain non-homologous chromosome segments of *Pisum* containing centromeres or knobs is only a suggestion which at present lacks the support of more thorough investigations.

Chromosome 2

The two translocations involving chromosome 2, *i.e. T(1—2)a* and *T(1—2)b*, both show close linkage between the point of interchange and the gene *A*. Cross No. 2 of Table 3 b shows that the point of interchange in the former translocation is probably situated between the genes *A* and Vi_1. According to PELLEW (1940) there is about 1 % of crossing over between *A* and the translocation point, but the segregation of 122 F_2 plants given on p. 376 of her paper give the value 3.8 ± 2.5 % which is in good agreement with the value 4.2 ± 1.27 % of Table 3 b.

According to Table 3 b (cross No. 3—4) there is also close linkage between *A* and the translocation *T(1—2)b*. This segregation has been studied by PELLEW (unpubl.) in F_2 progenies from crosses between structural type 9 (see SANSOME, 1938) and the normal structural type.

A third case involving chromosomes 1 and 2 has been studied by LAMPRECHT (1955 c). According to our nomenclature the translocation involved in his Line 21 (between chromosomes VII and I) should be symbolized as *T(1—2)c*.

The cases mentioned above show that chromosome 2 contains the marker gene *A*.

Chromosome 3

Translocations involving chromosome 3 show in each case linkage between their point of interchange and the genes *St* and *B* (*cf.* LAMM, 1948, 1951, 1956). The linkage values in progenies of translocation heterozygotes involving chromosome 3 are given below.

Translocations names	symbols	Marker genes	Linkage values	References
Thibet 7	*T(1—3)a*	*St*	27.0 ± 3.30 %	LAMM, 1951
„	„	*B*	1.0 ± 1.06 „	„ „
Extra Rapid N	*T(1—3)b*	*St*	8.0 ± 0.96 „	„ 1948
„ „ „	„	*B*	17.5 ± 1.72 „	„ „
Merton 1	*T(3—4)a*	*St*	4.1 ± 2.97 „	Table 3 b
„	„	*B*	8.5 ± 4.32 „	„ „
NILSSON's NIII	*T(3—5)a*	*St*	22.4 ± 5.91 „	NILSSON, 1950
„ „	„	*B*	8.2 ± 3.87 „	„ „

The linkage between *T(1—3)b* and *F* found in the present study (see cross No. 15 of Table 3 b) offers additional support to the previous evidence that the chromosome containing *B* and *St* (*cf.* LAMPRECHT, 1953) is the one involved in the translocation. The gene *M* of the same chromosome is, as might be expected, too far from *T(1—3)b* to give any sign of linkage (see cross No. 16 of Table 3 b).

In cross No. 24 between Merton I and the normal structural type an unexpected linkage between *T(3—4)a* and *R* has been detected (see Table 3 b). This linkage, although significant, is probably accidental. Similar exceptions have been found for instance in barley where an apparent close linkage in F_2 was not confirmed in F_3 (BURNHAM and HAGBERG, 1956).

There is disagreement between the results of the present study and those of the investigation made by NILSSON (1950) concerning the linkage between the point of interchange in *T(3—5)a* and the gene *St* (*cf.* cross No. 26 of Table 3 b). NILSSON (1950) also found linkage between *Wlo* and the point of interchange in this same translocation but no such linkage was found in the present investigation (see cross No. 26 of Table 3 b). Possibly these discrepancies are due to the presence of a deviating structural type in Line 88 (our line of NILSSON's NIII), for in one hybrid

plant between Line 88 and Line 111, *i.e.* in the supposed cross *T(3—5)a*×*T(3—4)a* two rings of four were observed at MI instead of the expected ring of six which was found in other hybrids of the same combination (*cf.* Table 2).

Chromosome 5

The linkage between *T(3—5)a* and *Le* in the crosses Nos. 25—26 of Table 3 b amounts to 22.4±3.34 %, which is in good agreement with earlier results obtained by NILSSON (1950). The gene *Lat* of the same chromosome (*cf.* LAMPRECHT, 1958 a) does not give linkage with *T(3—5)a* (see Table 3 b), which might be expected with regard to the considerable distance between *Le* and *Lat*. Slight linkage between *T(3—5)a* and the genes *N* and *Fa* of chromosome 5 was found by NILSSON (1950). No linkage has been found between *T(5—6)a* and *Le* (see crosses Nos. 27—31 of Table 3 b). Since *Le* cannot possibly belong to chromosome 3 it must be situated in chromosome 5.

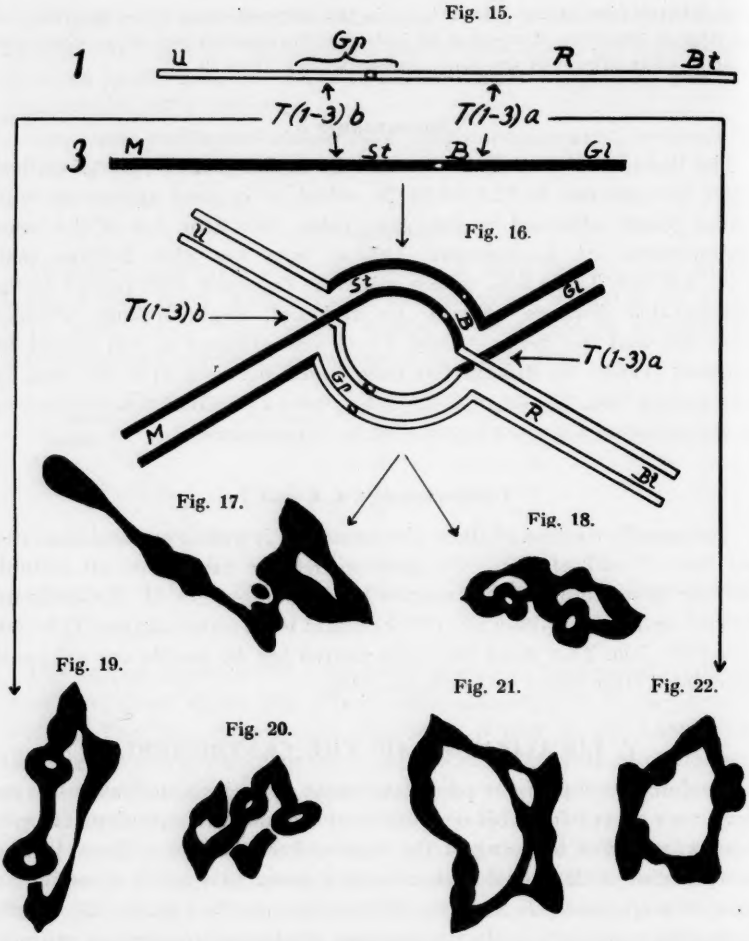
Chromosomes 4, 6 and 7

The genetic content of these chromosomes is unknown, and the crosses Nos. 27—37 of Table 3 a give no definite hints. For all suitable marker genes situated in Lamprecht's chromosomes II (LAMPRECHT, 1955b) and VI (LAMPRECHT, 1957b) ought to be tested against *T(5—6)a* and *T(6—7)a*. This work has been started but no results are yet available apart from those in Table 3 a—3 b.

V. LOCALIZATION OF THE CENTROMERES

To date fixation of the pachytene stage of meiosis in *Pisum* has not been very successful. This occasions considerable inconvenience in connection with the mapping of the centromeres. However, the cytogenetical studies of the translocation heterozygotes have given some indication of their probable location. With reference to Figures 15—25 the following comments as to the location of the centromeres in chromosomes 1 and 3 will be given.

According to LAMPRECHT (1948) the centromere of chromosome 3 (=LAMPRECHT's chromosome III) is probably situated between the genes *M* and *St* (see Fig. 15). This is, however, incompatible with the unaltered linkage relations of the *St-B* segment in the *T(1—3)a* translocation heterozygote (*cf.* LAMM, 1951) and the linkage reduction of the same segment found in the *T(1—3)b* heterozygote (*cf.* LAMM, 1948). Furthermore, if LAMPRECHT were correct the *T(1—3)a* heterozygote



Figs. 15—22. Meiosis in translocation heterozygotes involving *T(1—3)a* and *T(1—3)b*. — Fig. 15. Diagram to show the probable constitutions of chromosomes 1 and 3. Loci, centromeres and *T*-points are not accurately placed. Only their relative positions are shown. — Figs. 16—18. Pachytene diagram of the *T(1—3)b*×*T(1—3)a* heterozygote and two common types of resulting metaphase configurations. — Figs. 19—20 and 21—22. Rings-of-four from the *T(1—3)b*×normal and the *T(1—3)a*× ×normal translocation heterozygotes. Figs. 21—22 also demonstrate figure-of-eight configurations due to chiasmata in an interstitial segment.

Figs. 23—25. Microphotos of complete nuclei at MI corresponding to the configurations illustrated by Figs. 19, 17 and 22.

would then contain two long interstitial segments, whereas the metaphase configurations only indicate the existence of one such segment (see Figs. 21, 22 and 25). This interstitial segment must, however, belong to the *Gp-R* segment of chromosome 1 as well be discussed later.

Probably the centromere of chromosome 3 is situated within the *St-B* segment as indicated by Figure 15 or perhaps slightly to the right of this segment. However, if the centromere were situated at least much to the right of *B* the *T(1—3)b* translocation heterozygote would give considerably more chiasmata in the interstitial segment than actually cytologically observed. Usually the rings-of-four are of the types illustrated by Figures 19—20 and a figure-of-eight, although sometimes occurring (*cf.* LAMM, 1951, Fig. 5), is quite rare.

In the *St-B* regions of chromosome 3 as well as in the *Gp* region of chromosome 1, the linkage variations are high (LAMPRECHT, 1948). In general the centromeric regions should be most variable in their crossing-over properties (MATHER, 1938).

In translocations involving chromosome 1, increasing distance between the point of interchange and *Gp* and decreasing distance between this point and *R* is associated with an increasing tendency of figure-of-eight formation due to chiasmata in the interstitial segment. In translocation heterozygotes between the normal structural type and *T(1—2)a* or *T(1—3)b* there is close coupling between *Gp* and the point of interchange (*cf.* p. 428). Here the ring-of-four formation is quite regular. In *T(1—3)a* and *T(1—4)a* translocation heterozygotes, on the other hand, the figure-of-eight type is common (*cf.* Figs. 8, 21, 22 and 25) and the coupling between the point of interchange and *Gp* is less close (*cf.* p. 428). Hence *Gp* is more proximal to the centromere than *R*.

The slight or entirely failing linkage between the point of interchange

and the gene *R* in the *T(1—3)b* heterozygote might be explained by the suggested position of the centromere in chromosome 1 (see Fig. 15). Whether the gene *Gp* is slightly to the right or to the left of this centromere is at present difficult to assess.

Strong support in favour of the centromere location of Fig. 15 is also found in the peculiar metaphase configurations observed at meiosis in the hybrid *T(1—3)a*×*T(1—3)b* (Figs. 15, 16, 17, 18 and 24, see also LAMM, 1951, Figs. 8—12). This hybrid probably corresponds to the type 1 a, given by GOPINATH and BURNHAM (1956).

In *T(1—2)a* the gene *Gp* of the former and *A* of the latter chromosome are both very near the translocation point. In hybrids with the normal structural type interstitial chiasmata do not occur in the ring-of-four giving rise to a figure-of-eight (*cf.* HÅKANSSON, 1931; SUTTON, 1939). In *T(1—2)a* two new chromosome types have been created (SANSOME, 1933) of which one is considerably longer and one correspondingly shorter than either of the non-interchanged chromosomes. Probably the point of interchange is situated in the proximal segments of the long arm of chromosome 2 and the sohrt arm of chromosome 1.

The classical "figure-of-eight" in *Pisum* described by SANSOME (1932 and 1933) in the hybrid *T(1—3)a*×*T(1—2)a* is in good agreement with the centromere and interchange positions given in the pachytene scheme of Fig. 26, whereas the metaphase configurations described by LAMM (1951, Figs. 14—15) for the hybrid *T(1—3)b*×*T(1—2)a* are explained by the scheme of Fig. 27. In the latter case no chiasmata are formed in the rather short differential segment.

The final localization of the centromeres would require the completion of linkage studies within the different structural types. So far this has only been partially done within structural homozygotes of *T(1—3)a* (*cf.* LAMM, 1951). If, however, only one of the centromeres could be definitely localized and still more translocations were available, it would not be too difficult to continue the mapping of the other centromeres by the methods given by ANDERSSON and RANDOLPH (1945).

VI. DISCUSSION

A notable contribution to the present investigation of the cytogenetical studies in *Pisum* is the establishment of a "basic" translocation tester set involving each of the seven chromosomes. This tester set has been cytologically checked by meiotical analysis of the pairing behaviour in F_1 hybrids of dialled crosses. In addition to the translocations involved a number of other translocations have been described

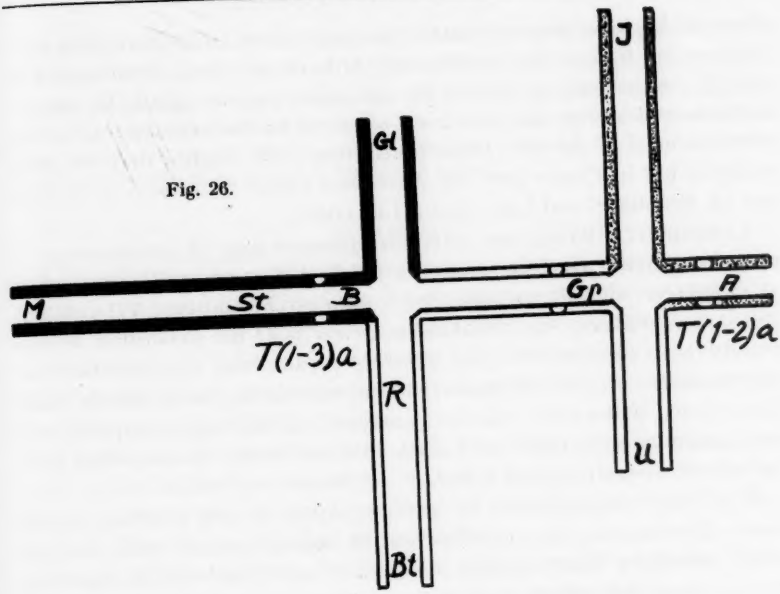


Fig. 26.

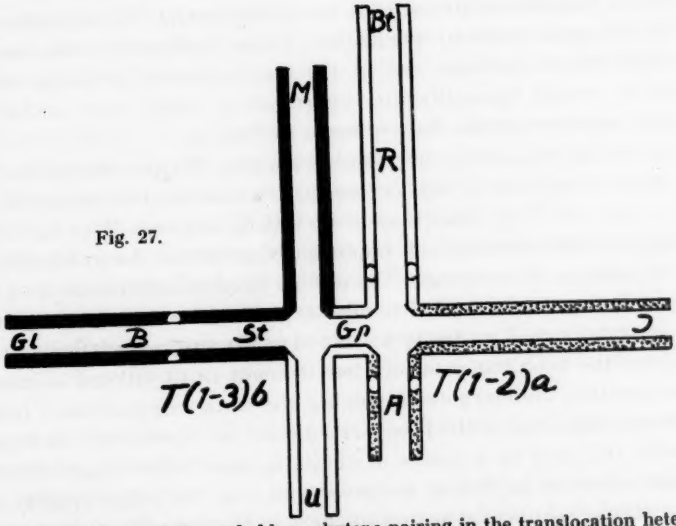


Fig. 27.

Figs. 26—27. Diagrams of the probable pachytene pairing in the translocation heterozygotes *T(1—3)a*×*T(1—2)a* and *T(1—3)b*×*T(1—2)a*. In the former, a figure-of-eight is often formed due to chiasmata in the differential segment, whereas open rings of six are formed in the latter. Loci, centromeres and *T*-points are not accurately placed. Only their relative positions are shown.

above all by LAMPRECHT in numerous papers, but since the stocks have not been exchanged the results have hitherto not been coordinated. It should also be mentioned that the cytological studies made by the co-workers of LAMPRECHT have been confined to the examination of the chromosomes at mitosis. Deductions from such studies may be quite valuable but in *Pisum* they are sometimes rather hazardous as pointed out by MORRISON and CHU-CHANG LIN (1955).

LAMPRECHT (1957 a) has given an extensive map of chromosome V, containing inter alia the genes *Gp-Cri-Fs-Ust*; and two years earlier (LAMPRECHT, 1955 d) he published a map of chromosome VII containing, among others, the well-known genes *R-Tl-Bt*. According to our results these chromosomes are different segments of one and the same chromosome, *viz.* chromosome 1. This conclusion could hardly have been drawn if the pure genetical analysis had not been complemented by meiotic investigations (*cf.* LAMM, 1951 and 1956). A somewhat similar case has been revealed in barley (*cf.* BURNHAM, 1956 b).

If, as has been shown in the present paper, *Gp* and *R* belong to the same chromosome, the co-ordination of linkage groups with chromosomes made by BLIXT (1955) and GELIN and BLIXT (1956) must be wrong. Since chromosome 1 will possess the gene order *Ust-Fs-Gp-R-Bt*, one would imagine that LAMPRECHT's Line 680 (LAMPRECHT, 1955 a) perhaps contains an inversion of this chromosome. The objections given by LAMPRECHT (1958 b) would then not be valid and, furthermore, the segment *Gp-R* and also one of the chromosomes (probably chromosome 4) would exemplify the occurrence of large, more or less gene-empty segments in the chromosomes of *Pisum*.

According to LAMPRECHT (1955 d) the gene *Wsp* is situated to the left of the *R-Bt segment*. If this is true there would be close linkage between *T(1—3)a* and *Wsp*. Since there is no linkage between *Wsp* and *Gp* such a result would constitute an important support for the independence of LAMPRECHT's chromosomes V and VII. Crosses between *T(1—3)a* and a *wsp*-line of the normal structural type have been performed, and the F_2 segregation will probably be scored in the summer of 1960.

From the breeding point of view it might be of interest to intercross translocation homozygotes involving the same chromosomes but with different positions of the breaks. As shown by GOPINATH and BURNHAM (1956), this may be a means of obtaining new fertile types with duplicated segments. In *Pisum* we know that, *e.g.*, the edible quality of the *rr* genotype is superior to that of the *RR*. Would perhaps a strain only duplicated for *rr*, but otherwise diploid, be still further superior? From

this point of view it would be worth while to make crosses between *T(1—2)a* of the basic tester set and LAMPRECHT's *T(1—2)c* (see p. 430).

Acknowledgements. — The present investigation has been financially supported by the NILSSON-EHLE foundation. We also wish to express our indebtedness to Professor F. NILSSON for providing us with facilities to carry out the *Pisum* investigations at the Horticultural Research Station at Alnarp.

SUMMARY

A "basic" tester set containing five translocations involving all of the seven chromosomes in *Pisum* has been collected and checked by cytological analysis of the chromosome pairing at meiosis in the F_1 hybrids of dialled crosses. The translocations of this tester set have short interstitial segments and the pairing behaviour at meiosis of the translocation heterozygotes is fairly simple.

In addition, three translocations with long interstitial segments have been studied. Since the interstitial segments are effective in the reduction of crossing-over values these types of translocations may be particularly valuable in genetical mapping work.

Genetical investigations of the linkage relations between the points of interchange and certain marker genes have shown that:

(1) Chromosome 1 carries the genes *Gp* and *R*. According to LAMPRECHT (1957 a and 1955 c) these genes are situated in different chromosomes, *viz.*, chromosomes V and VII of his chromosome maps. The present investigation, however, constitutes strong evidence against this assumption.

(2) Chromosome 2 carries the gene *A* and corresponds to LAMPRECHT's chromosome I (LAMPRECHT, 1956).

(3) Chromosome 3 carries the genes *St* and *B* and corresponds to LAMPRECHT's chromosome III (LAMPRECHT, 1953).

(4) Chromosome 5 carries the gene *Le* and corresponds to LAMPRECHT's chromosome IV (LAMPRECHT, 1958 a).

(5) The genetic content of chromosomes 4, 6 and 7 has yet not been determined. Two of these chromosomes probably correspond to chromosome II (LAMPRECHT, 1955 b) and chromosome VI (LAMPRECHT, 1957 b).

The probable location of the centromeres belonging to chromosomes 1, 2 and 3 has been discussed.

The probable occurrence of quasi-linkage, perhaps due to interaction between certain chromosomes across the cytoplasm, has been considered.

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ADDITIONAL EXPERIMENTS ON IRRADIATED POPULATIONS OF DROSOPHILA MELANOGASTER

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(Received February 2nd, 1959)

IN a previous paper (BONNIER, JONSSON and RAMEL 1958) experiments were described on the effect of selection pressures of different strengths on x-ray irradiated populations of *Drosophila melanogaster*. The preparations for these experiments began in the middle of 1956 and the first irradiations were made in Dec. of that year. The published paper included the results up to about the end of 1957, but the experiments themselves were prosecuted also after that time. In the spring of 1958 the results from the different populations were found to be consistent enough to make it possible to formulate some tentative assumptions as to their explanations. (These assumptions were presented at the Geneva meeting on the peaceful uses of atomic energy, Sept. 1958.) It seemed then to be more profitable to start a new set of experiments — designed with the purpose of testing the validity of the assumptions made — than to produce still more generations with the populations described in the previous paper. These earlier experiments were, accordingly, discontinued during the spring and summer of 1958.

All of the populations of the previous paper started from strains which were made homozygous and isogenous for the three major chromosomes. Their viabilities decreased during the earlier generations; this was especially marked in the populations kept on a low selection pressure; in fact, in one of them, a catastrophe of extinction was avoided at a very small safety margin. The populations gained, however, considerably in viability with increasing number of generations, and with the concomitantly increasing accumulated irradiation dose on the chromosomes and degree of heterozygosity. In this way they reached a viability level well above that prevailing at the homozygous start. The tentative assumptions, spoken of above, were based on such facts. In the present

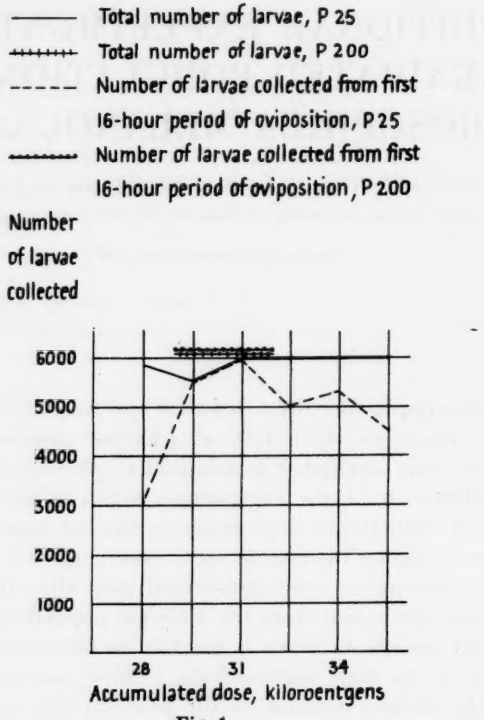

Fig. 1.

article we are not going to take up any discussions — these must await more results to be available from the new set of experiments. Our purpose is now only to fill up the gap between the results described in our previous paper and the moments of discontinuing the experiments with these earlier populations. In order to make the presentation of these additional data as short as possible we are using the same symbols as in the previous paper. The present article should, therefore, be read in connection with the previous one, where also all details of the methods used are to be found. When referring to figures, tables, or notations of that paper the letter "B" will be used in the present article.

The continuation of the experiments shown in B, Figs. 4 and 5, are given in Figs. 1 and 2 respectively. As may be seen all the populations remained on a high viability level.

In B, Table 6, the results of analyses of lethal 2nd chromosomes

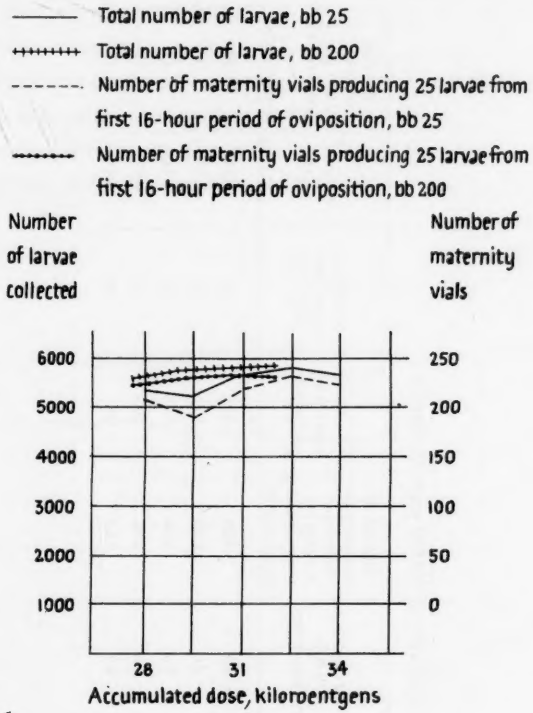

Fig. 2.

were given. At one more occasion such analyses were made, the results of which are shown in Table 1. There is a faint indication that in populations with low selection pressure, P 25 and bb 25, the average number of lethal genes in lethal chromosomes will accumulate at a higher rate than in populations with strong selection pressure, P 200, bb 200, R.

As described in the previous paper (p. 403) experiments with two new populations were begun in January 1957. One of these, bb 25—c, was at the start made homozygous and isogenous for the three major chromosomes, but unrelated to the foundation strain of the other populations. The second population, bb 25—d, was started with flies from bb 200 which then had reached a high viability level. Both of these populations were, as indicated by their names, run on low selection pressure and with a check that individual females did not contribute more than a limited number of progeny for the next generations. The

TABLE 1. *Analysis of lethal chromosomes.*

Population	Accumulated dose, kiloroentgens	Number of chromosomes tested in homozygous condition	Number of chromosomes lethal when homozygous	Percent lethal chromosomes	Number of heterozygous crosses performed between lethal chromosomes	Number of lethal genes observed	Number of lethal loci observed	Spread index	Average number of lethal genes observed in lethal chromosomes
P 25	28.0	50	31	62	465	27	39	1.44	1.26
P 200	27.5	64	36	56	630	27	36	1.33	1.00
bb 25	28.0	64	45	70	990	33	64	1.94	1.42
bb 200	27.5	65	49	75	1176	35	55	1.57	1.12
R	27.5	65	40	62	780	28	46	1.64	1.15

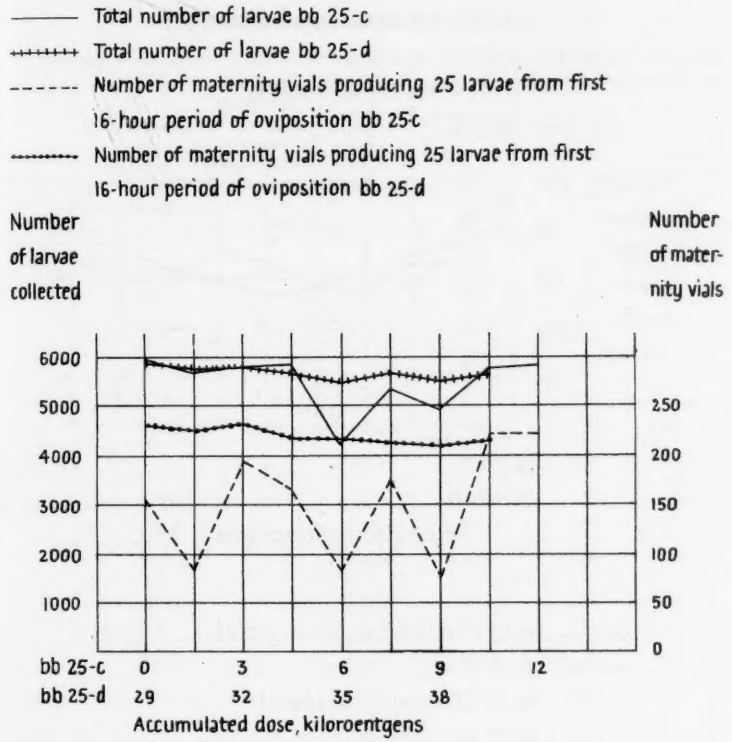

Fig. 3.

accumulated dose to the chromosomes was at the start nil for bb 25—c but 27.5 kiloroentgen for bb 25—d.

The first results, corresponding to diagram B, Fig. 5, were given in B, Table 8. The continuation of these data are now shown in Fig. 3. The difference in viability is clearly seen when comparing the two curves which show the number of maternity vials producing 25 or more larvae from the 1st 16 hour period of oviposition.

The egglaying capacity and the hatchability of the eggs was, for the earliest generations, compared in B, Table 7. The full results are now shown in Figs. 4 and 5. The tests were made here both with flies which themselves were non irradiated as well as with flies which themselves were irradiated. No clear differences between the populations were observed with regard to hatchability; likewise no differences could be

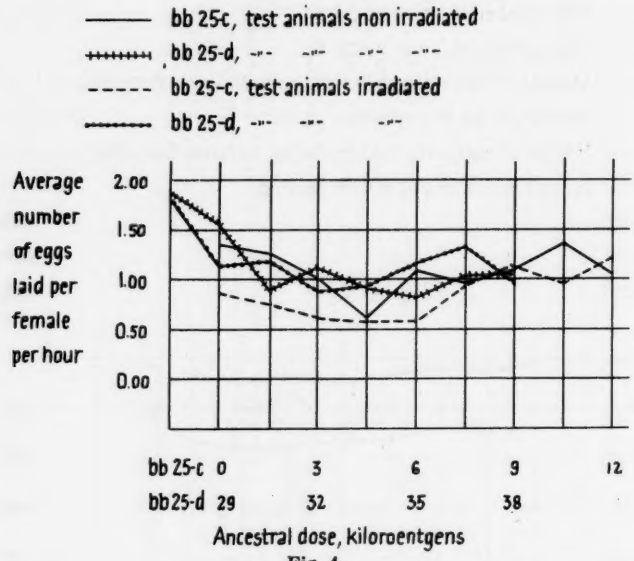
bb 25-c, test animals non irradiated
bb 25-d,
bb 25-c, test animals irradiated
bb 25-d,
Average number of eggs laid per female per hour
2.00
1.50
1.00
0.50
0.00
bb 25-c 0 3 6 9 12
bb 25-d 29 32 35 38
Ancestral dose, kiloroentgens

Fig. 4.

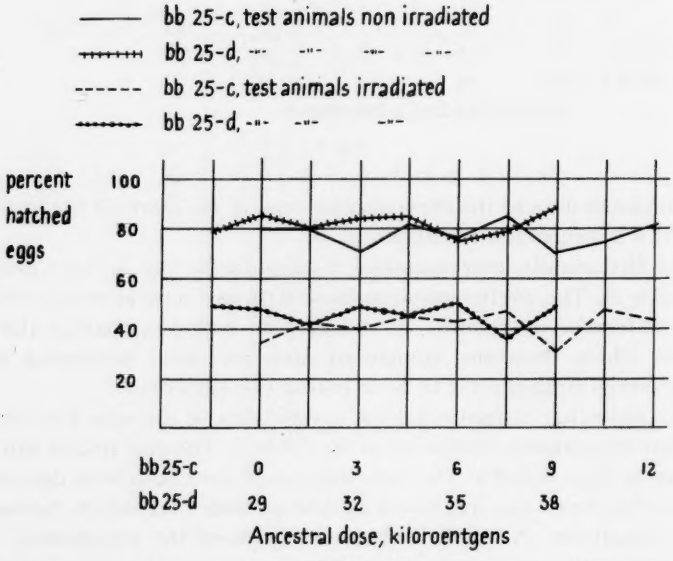
bb 25-c, test animals non irradiated
bb 25-d,
bb 25-c, test animals irradiated
bb 25-d,
percent hatched eggs
100
80
60
40
20
bb 25-c 0 3 6 9 12
bb 25-d 29 32 35 38
Ancestral dose, kiloroentgens

Fig. 5.

TABLE 2. *Frequency of sterile males.*

(The letters *p* are symbols used for the comparisons in the bottom of the table)

Populations	Accumulated dose from ancestral generations, kiloroentgens	Irradiation of the tested males with 1.5 kiloroentgens: 0 = not irradiated 1 = irradiated	Original number of males	Number of males which were alive during the whole of the test	Number of sterile males among the alive males	Percent sterile males	
bb 25—c	0—6.0	0	300	280	18	6.4	*p1*
	7.5—13.5	0	250	236	28	11.9	*p2*
	Total	0	550	516	46	8.9	*p3*
	0—6.0	1	250	237	28	11.8	
	7.5—13.5	1	250	231	28	12.1	
	Total	1	500	468	56	12.0	*p4*
Total			1050	984	102	10.4	*p5*
bb 25—d	29.0—33.5	0	200	186	22	11.8	
	35.0—41.0	0	250	233	27	11.6	
	Total	0	450	419	49	11.7	*p6*
	27.5—33.5	1	250	235	37	15.7	
	35—41.0	1	250	238	35	14.7	
	Total	1	500	473	72	15.2	*p7*
Total			950	892	121	13.6	*p8*

Probabilities for comparisons: *p* 3—*p* 4 significance P>0.11
p 6—*p* 7 „ P>0.12
p 1—*p* 2 „ P 0.03—0.04
p 5—*p* 8 „ P 0.03—0.04

demonstrated between egglaying capacities when the test animals were themselves unirradiated. However, for all of the earlier generations, the homozygous — or later, less heterozygous — females of population bb 25—c were, with regard to their egglaying capacities much more sensitive to radiation than the more heterozygous females of population bb 25—d.

On B, p. 395, a description is made on the method used for testing male sterility and some data concerning the frequency of such males is given in B, Table 3. More tests on male sterility were later made with males from the two populations bb 25—c and bb 25—d. These tests were made both on males which were themselves unirradiated as well

as on males which were themselves irradiated. The results are presented in Table 2. In the case of bb 25—c the males were taken from generations covering ancestral doses from 0 to 13.5 kiloroentgens; the corresponding range of doses is, in the case of bb 25—d, 27.5 to 41 kiloroentgens. These data are too small to permit comparisons of changes from generation to generation. In Table 2 we have, therefore, grouped the generations into two groups; and this is made both with regard to unirradiated and irradiated males. Males which themselves were irradiated show an averagedly higher frequency of sterility than do those which were unirradiated (comparisons $p\,3$—$p\,4$ and $p\,6$—$p\,7$ of Table 2). The differences are, however, not significant and may thus, at the very most, be taken as indicative. Somewhat more reliable are the differences in frequency of sterility when comparing high v. low accumulated dose of ancestral irradiation. (Comparisons $p\,1$—$p\,2$ and $p\,5$—$p\,8$ in Table 2.) If these differences are really true, Table 2 also indicates that the frequency of male sterility does not increase after the ancestral dose having reached a certain level (comparisons within bb 25—d between the two groups of generations, i.e. between the two differences 11.6—11.8 and 14.7—15.7).

Acknowledgements. — The authors are indebted to the Swedish Atomic Energy Commission and the Swedish Natural Science Research Council which have supported this investigation.

SUMMARY

This article follows up results from experiments, the earlier parts of which, were described in a previous paper. The additional data of the present article are in full agreement with the earlier ones.

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THE CHROMOSOMES OF THREE HUMAN CELL STRAINS

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MATERIAL AND METHODS

CELL strains well-adapted to permanent growth in tissue culture are characterized by chromosome conditions essentially different from those of normal cells. Instead, they show striking resemblances with old transplantable tumors. Thus, their chromosome complement varies from cell to cell. These variations, involving both number and structure, fluctuate around one stemline karyotype, which usually differs from the normal karyotype of the species. These facts indicate that the cell strain genotype has undergone, during its history, a far-reaching reorganization making it well-adapted to the new life habit of growth *in vitro*.

In the present work the chromosomes were studied in three human cell strains, two of which were of normal origin and the third of cancer origin. All three strains were grown serially at the tissue culture laboratory of the Institute of Bacteriology, Lund.

The following strains were studied:

1. Fogh. This strain, established in March 1956 at the University of California, Berkeley, from human amnion (FOGH and LUND, 1957), has been carried at the Institute of Bacteriology, Lund, since March 1958. It has passed 120—150 transfers at Lund.

2. Parotis, started in October 1957 by Dr. SVEN BERGMAN from parotis glands of a five months old, male embryo, has gone through more than 50 passages (cf. BERGMAN, 1959, and BERGMAN, NILSSON and OLSSON, 1959).

3. H. Ep. 1. This was an epidermoid carcinoma of the cervix of a 52 years old female. It was established in tissue culture in February 1953 at the Sloan-Kettering Institute, New York (FJELDE, 1955, 1956). This tumor exists in different strains. The strain studied by me was brought from Sloan-Kettering to Copenhagen in 1953 by Dr. FJELDE,

who delivered it to the Institute of Bacteriology at Lund in June 1958. A second line of H. Ep. 1 was carried at the Sloan-Kettering Institute *in vitro* (MOORE, SABACHEWSKY and TOOLAN, 1955) and in animal hosts (TOOLAN, 1953, 1954). Material of this second strain was used for chromosome studies by LEVAN (1956, and unpublished).

TCM 199 was used as culture medium with 20 % human serum for outgrowth, and with 2 % calf serum for maintenance of the cells. All media were given 2 % tryptos (Difco), and 100 units penicillin, 100 γ streptomycin and 10 units mycostatin were added per ml medium.

After having been loosened from the glass wall by one or two minutes of trypsin treatment the cells were transferred, suspended in medium, to horisontally oriented slides, on which they were kept for about 24 hours within an agar frame. After the cells had sedimented onto the glass surface and become attached to it, the slides were placed in perpendicular position in ordinary glass jars used for staining, each glass jar holding 50 cc medium. In 3 to 14 days at 37 centigrades and CO_2 containing atmosphere the cells will grow out into a dense monolayer covering one side of the submersed part of the slide. This method, developed by Dr. BERGMAN, yielded slides very favorable for chromosome studies.

After it has been found by microscopic inspection that one or two slides of a jar show good cell growth, the rest of the slides are taken for chromosome study. The procedure is as follows: 5 cc 0.0005 % colchicine is added to every 50 cc medium, making it 1.25 μ Mol/l for colchicine. After one to two hours the slides are transferred, one or two at a time, into hypotonic (1.12 %) sodium citrate. There is a tendency of the cells to fall off from the slide during this treatment. By shortening the time of hypotonic treatment to 4—7 minutes this difficulty is avoided.

Now, each slide is fixed and stained in acetic orcein (2 % orcein in 60 % acetic acid). It is important to place the coverslip in position immediately to avoid losses of cells, which are loosening from the glass surface in the acetic. The staining is controlled under the microscope, and the cells are squashed, as soon as the chromosomes have taken good stain.

This technique gave very favorable slides permitting exact chromosome number counts. Fifty counts were made for each strain, a free hand drawing being made for each count; two to four idiogram analyses were made of each material. Each chromosome was depicted with the aid of a camera lucida under high power in the center of the view

field. This gave drawings at 6100 times magnification, from which chromosome analyses and measurements were made.

OBSERVATIONS

1. Fogh. This strain, showing very good growth, was in optimal condition after seven days. At that time its mitotic frequency was often high. Its chromosomes were studied on slides fixed on June 16, 30, and July 2, 1958.

The chromosome numbers, recorded in Table 1 and Fig. 1 *a*, varied from 72 to 82 with the stemline number 76, thus a hypertriploid stemline ($s=3x+7$). In addition, cells were seen with 152 to 160 chromosomes, and also considerably higher numbers. They represent $2s$ and higher multiples of the stemline number. Among 500 cells 5.8 % were $2s$ or higher. Endoreduplications (LEVAN and HAUSCHKA, 1953), observed a few times, may be the cause of these higher numbers.

Two idiogram analyses were made and are pictured in Fig. 2. The chromosomes are divided into three classes according to the location of the centromere: 1. M chromosomes (centromere median, index long arm : short arm less than 2), 2. S chromosomes (centromere subterminal, index 2 — less than 5), and 3. T chromosomes (centromere terminal, index over 5).

The stemline of the Fogh strain contained a majority of M chromosomes, the three classes M:S:T occurring in the proportion 45:19:14 in the two cells analyzed (Table 2, nos. 1, 2). The idiogram *c* of Fig. 2 is from a somewhat earlier mitotic stage than *d*, as seen from its low degree of contraction.

2. Parotis. This strain, too, had quite a good growth reaching a suitable stage for fixation after some 10 to 14 days. Cytologic analyses were made on slides fixed on August 19 and September 15, 1958.

Dr. BERGMAN has informed me that this strain permanently contains two morphologically different cell types, one epithelial-like, with rounded cells, and one more fibroblast-like, with an extended mode of growth. This heterogeneity in cell type is in accord with the observation that this cell strain has two stemlines, one having 73, the other 79 chromosomes, both thus being hypertriploid ($3x+4$ and $3x+10$, respectively). The chromosome number extremes of the stemline region were 70 and 82. That the two stemlines were well defined is clear from Table 1 and from the bimodal histogram of Fig. 1 *b*.

A considerable number of cells were found to have chromosome num-

TABLE 1. *Chromosome numbers of the three cell strains.*

Cell strain	Chromosome number															n	*s*	M
	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82			
Fogh	—	—	—	—	1	3	4	7	18	9	4	2	1	—	1	50	76	76.1
Parotis	—	—	2	1	4	12	5	2	2	3	4	9	3	2	1	50	73.79	75.7
H. Ep. 1	1	3	7	6	20	4	5	2	2	—	—	—	—	—	—	50	72	71.9

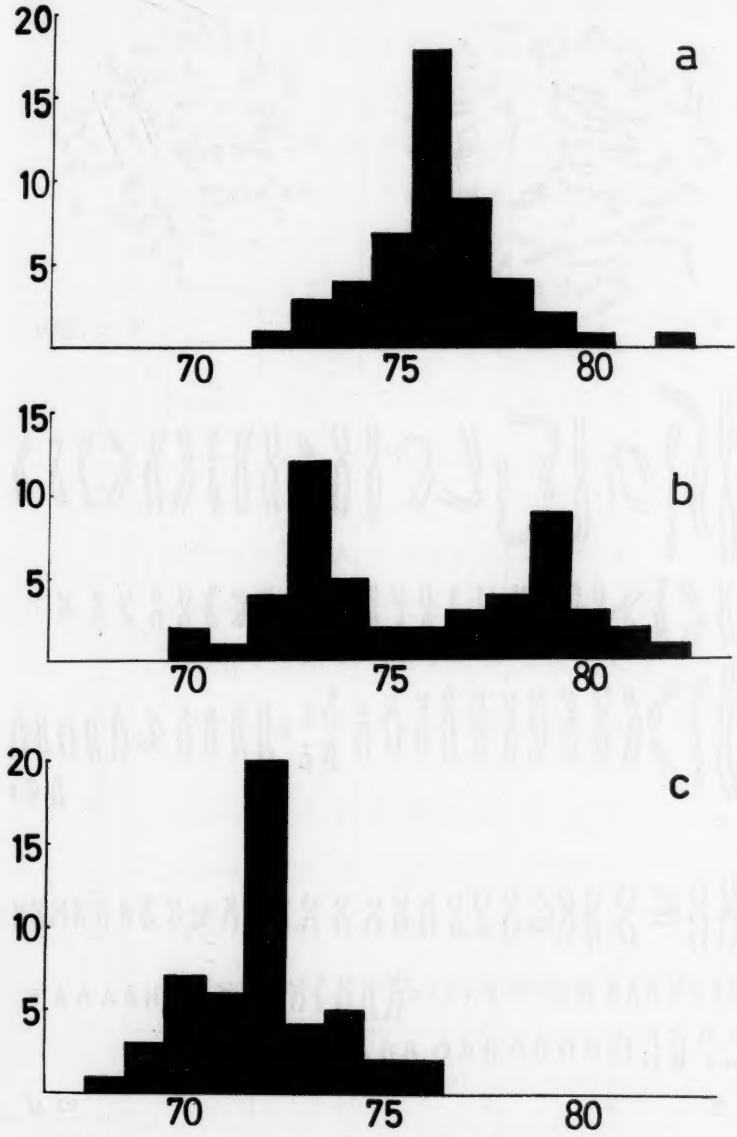

Fig. 1. Histograms showing the chromosome number distribution for a: Fogh, b: Parotis, c: H. Ep. 1.

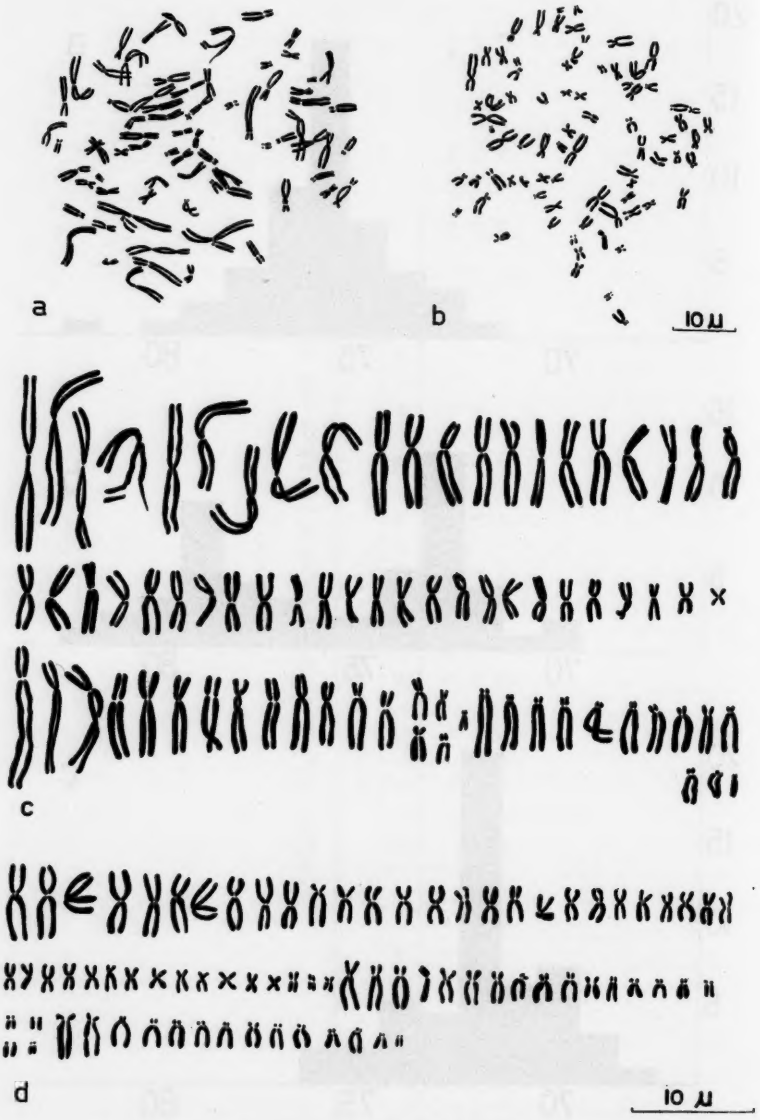

Fig. 2. Idiogram analyses of Fogh (a, b, ×1080; c, d, ×1900).

TABLE 2. *Length variation within the three chromosome types.*

Cell strain	Idiogram no.	M				S				T				Total			
		Number	Average length	Shortest	Longest	Number	Average length	Shortest	Longest	Number	Average length	Shortest	Longest	Number	Average length	Shortest	Longest
Fogh	1	46	60	15	143	18	55	15	113	13	36	16	33	77	51	15	143
	2	43	39	10	65	20	20	8	39	14	20	7	38	77	26	7	65
	Average	45	50	13	104	19	38	12	76	14	28	12	46	77	39	11	104
Parotis	3	39	43	10	85	24	55	15	62	9	27	14	34	72	38	10	85
	4	44	35	13	79	25	32	15	50	10	17	8	24	79	25	8	79
	5	42	48	16	96	28	40	19	68	9	27	13	58	79	38	13	96
	6	85	37	13	84	51	34	20	54	16	23	6	31	152	31	6	84
	Average	42	41	13	86	26	40	17	59	9	24	10	37	76	33	9	86
H. Ep. 1	7	35	37	9	75	28	37	14	62	8	25	18	32	71	33	15	50
	8	38	29	12	73	28	33	16	60	8	17	9	21	74	26	13	44
	9	37	42	10	101	27	39	14	71	8	17	7	35	72	33	10	56
	Average	37	36	10	83	28	36	15	64	8	20	11	29	72	31	13	50

Fig. 3. Idiogram analyses of Parotis (a, b, s=79; c, d, s=73), and H. Ep. 1 (e, f). (a, c, e, ×800; b, d, f, ×1500).

bers between 146 and 156, thus at the 2*s* numbers. Among 500 cells 16 % had 2*s* or higher numbers.

The four idiogram analyses made are recorded as nos. 3—6 in Table 2. They represent the 73 stemline (no. 3), the 79 stemline (nos. 4, 5), and the 2*s* region (no. 6). The three chromosome types M:S:T occurred in the proportion 42:26:9 (Table 2). The ten longest chromosomes belonged to the M type. An idiogram from each stemline number is given in Fig. 3 b and d.

3. H. Ep. 1. This strain, being new in the tissue culture laboratory at Lund, had a decidedly slower growth than the preceding two strains. It formed mats only occasionally, the cells sticking loosely to the glass surface and thus often being lost during the hypotonic treatment. According to Dr. FJELDE (personal communication) this strain has exhibited vigorous growth in her laboratory at Copenhagen. It has become excellently adapted both for growth on the glass wall and in suspension. Chromosomes were studied on slides fixed on June 23, 26, July 5, and 23, 1958.

As in the two other strains the stemline number was hypertriploid. The relatively narrow chromosome number distribution was between 68 and 76 with a predominant stemline at 72 ($3x+3$ Table 1 and Fig. 1 *c*). This strain has only few cells with higher ploidy numbers. Among 500 cells 2.3 % had near-2*s* numbers, no higher numbers being seen.

Three idiograms were analyzed, the results being fairly uniform. The three chromosome types M:S:T occurred in the proportion 37:28:8 (Table 2, nos. 7—9). Satellites were noticed several times; no definite satellite chromosome was identified, however. One of the idiograms is pictured in Fig. 3 *f*.

DISCUSSION

All three cell strains studied agree in having a hypertriploid stemline distribution. H. Ep. 1 had the lowest stemline level, at 72, while Fogh had $s=76$. Parotis is of specific interest with its two stemlines, at 73 and 79. Any differences in chromosome behavior between the two materials with normal origin, Fogh and Parotis, and the cancer cell strain, H. Ep. 1, have been levelled during the long transplantation history of the three strains. The normal strains that supposedly started out at their explantation with the normal chromosome number 46, have obtained stemlines in the same chromosome number region as H. Ep. 1. The original chromosome number of H. Ep. 1 at its establishment *in vitro* is not known.

It is an interesting fact that the Parotis strain permanently maintains two distinct stemlines. Similar observations have been made in cancer materials. Thus, it was observed in a human ascites tumor (HANSEN-MELANDER, KULLANDER, and MELANDER, 1956) that two stemlines, at 58 and 63 respectively, persisted through the two occasions of fixation, ten days apart. Also the Sarcoma 37 ascites tumor of the mouse had at several fixations a fraction of near-diploid cells present, although the stemline was clearly at near-tetraploid level (LEVAN and HAUSCHKA, 1953).

In long-term cell strains, as well as in transplantable ascites tumors that have had time to reach good adaptation to their surroundings, one stemline number usually predominates, its genotype being of superior selection value within the cell population.

The question arises whether the Parotis strain, which was established more recently than Fogh and H. Ep. 1, was passing through an occasional labile period at the time of fixation. This seems hardly probable, considering that the two cell types persisted equally viable for at least one month and a half. On one occasion it was found that among 500 cells 47 % were epithelial-like and 53 % fibroblast-like. It is seen from Table 1 that about 48 % belonged to the stemline 79, and 52 % to the stemline 73. It was, however, not feasible to classify the cells according to growth type on slides stained for chromosome study, and *vice versa*, so nothing can be said as to possible relations of morphologic cell type to chromosome number level.

Because of the observed permanence of the two cell types, and of the two stemlines, in the population, it is likely that both types were equally well adapted to the prevailing environment. It may even be that each of the two cell types was favored by the presence of the other, that is, they may have reached a state of balanced equilibrium, a kind of symbiosis. Such a relation between different types of cells has been suggested earlier by SCHLEICH (1956), who was successful in growing tumor cells of the Yoshida sarcoma in tissue culture only in the presence of normal rat fibroblasts. She actually speaks of a symbiotic relation between the two cell types.

It should be mentioned that LEVAN studied tissue culture material of H. Ep. 1 at the Sloan-Kettering Institute in 1955, and again 1957 (LEVAN 1956, and unpublished). On both occasions the stemline number was found to be in the vicinity of 90, thus being decidedly higher than in the subline studied by me. As mentioned earlier, LEVAN studied another subline than the one studied here. Even though these different sublines

originate from the same tumor, their transplantation history is different. While the strain studied here never has been grown otherwise than *in vitro*, the strains carried at the Sloan-Kettering and studied by LEVAN have occasionally passed through cortisone-treated animal hosts.

SUMMARY

The chromosomes were analyzed in two human cell strains of normal origin (Fogh and Parotis) and one cell strain of cancer origin (H. Ep. 1). Chromosome counts were made in 50 cells of each and nine idiograms were drawn. All three cell strains had stemlines in the hypertriploid region ($s=72$—79). The Parotis strain was especially interesting, maintaining two stemline numbers through the period of observation, *viz.* 73 and 79. This strain is known always to contain two morphologic cell types, one epithelial-like and one fibroblast-like. The possible relations of the two stemlines to each other and to the morphologic diversity of this strain are discussed.

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BRIEF REPORTS

SVEN ELLERSTRÖM: The co-variation of chromosome number and environment in *Phleum pratense*.

(Received October 10th, 1958)

As is well known, naturally occurring polyploids, in many cases, show ecological requirements different from their corresponding diploids. In the case of allopolyploids this may be explained, as being a result of the co-operation between the different genotypes of the parental species.

To a certain extent, this explanation is valid also for the majority of natural occurring autopolyploids, which according to STEBBINS (1950) are supposed to be »intervarietal» or »intersubspecific» polyploids.

The creation of an autopolyploid in a more strict sense, however, does not give rise to any qualitative increase of the gene complement, in relation to the diploid parent. As most of the artificially produced polyploids of agricultural crops are autopolyploids of this type, it is of interest for plant breeders to know, to what extent the mere quantitative increase of the gene complement may change the ecological requirements of a plant. The present paper is a preliminary report of a study intended to elucidate this problem.

Among twin seedlings, occasionally formed in *Phleum pratense*, plants with the »polyhaploid» chromosome number $2n=21$ may be formed in a low frequency (MÜNTZING, 1937). Due to syncyte formation in the pollen mother cells of these plants, their offspring will show chromosome numbers ranging from $2n=35$ to $2n=84$ (LEVAN, 1941).

In an offspring from a crossing group consisting of seven »polyhaploid» plants, the euploid plants were selected for further propagation. The seed fertility of plants with $2n=56$ and $2n=70$ is almost as high as that of plants with $2n=42$.

The tillering capacity and dry matter yield in pure stand of these three »polyploid races», has been compared in sown field trials, located in different parts of the Scandinavian peninsula (*cf.* ELLERSTRÖM, 1959). Each field trial was arranged in a 4×4 Latin square, the four components being the 42-, 56- and 70-chromosome races, and a mixture of equal parts of these three polyploid races. The fourth component was made use of in a study of the competition between the polyploid races.

Table 1 summarizes the data on the density of the stand, measured as number of tillers in spring, in the trials at Svalöf (southern Sweden, latitude 55°35′), Ultuna (latitude 59°49′), and Notviken (northern Sweden, latitude 65°37′).

As is evident from the table, the 56- and 70-chromosome races have produced a larger amount of tillers in the trial at Notviken, relative to the 42-chromosome timothy, as compared with the other trials. These differences are statistically significant.

Table 2 gives the summarized data for the dry matter yield in the same trials. A statistical analysis of the data, reveals the fact that the three polyploid races react quite differently towards the diverse environments at the three testing places.

TABLE 1. *Number of tillers in three different trials with polyploid races of Phleum pratense.*

Locality of trial	Plot	Number of tillers	Relative values
Svalöf	42	123	100
	56	118	96
	70	75	61
	mixture	116	94
Ultuna	42	78	100
	56	75	96
	70	50	64
	mixture	67	86
Notviken	42	58	100
	56	67	116
	70	49	84
	mixture	60	103

(The figures give the mean values for number of tillers in a row of 0.5 metres length, determined at three different places in each of four plots and in three successive years.)

Using the 42-chromosome timothy as a standard, the 56-chromosome race is yielding less at Ultuna, and more at Notviken than at Svalöf. The 70-chromosome race is yielding significantly less than the standard in all three trials. However, in the first year ley, this difference is less marked at Notviken than at Svalöf. This agrees well with the results from the same type of trials at Tromsö (latitude 69°39′) and Tana (latitude 70°30′) in northern Norway. In these trials there is no significant difference in yield between the three polyploid races. The meteorological data gathered from the different testing places, together with the yield data here reported, and unpublished, indicate that the 56- and 70-chromosome races grow better under more humid conditions, relative to the standard. At the same time, the 56- and 70-chromosome races seem to grow better, relative to the standard, under the conditions prevailing in the northern part of the Scandinavian peninsula.

The high adaptiveness and the vigorous growth of normal, 42-chromosome, timothy under different environmental conditions is most probably, to some

TABLE 2. *Dry matter yield of polyploid races of Phleum pratense in three different trials.*

Locality of trial	Svalöf		Ultuna		Notviken	
Plot	Yield kg	Relative values	Yield kg	Relative values	Yield kg	Relative values
42	3.87	100	2.48	100	2.50	100
56	3.62	93	2.01	81	2.64	105
70	3.12	81	1.96	79	1.96	78
mixture	3.70	96	2.21	89	2.43	97

(The figures give the mean yield for three successive years.)

in his drawings. The fact that the kinetochore is divided at metaphase of extent, due to its hexaploid nature. Thus, it is less probable that an increase in chromosome number, above the hexaploid number, should result in an increase of vigour. However, in spite of the high polyploid nature of the material investigated, the results clearly show, that a quantitative increase of the gene complement considerably changes the reaction of timothy towards environment, and that the yield of the high polyploid races may, under certain conditions. at least equal that of the hexaploid timothy.

Even if these data are insufficient, for a more general conclusion, they nevertheless indicate the necessity, for the plant breeder, working with artificially produced autopolyploids, to test his polyploid material under a wider range of ecological conditions than the corresponding diploid material. This is in order to make full use of the advantages connected with the doubling of the chromosome number (*cf.* HAGBERG and ELLERSTRÖM, 1959).

Swedish Seed Association, Svalöf, Sweden.

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A. LIMA-DE-FARIA: Matrix and kinetochore in living material.

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The description of few chromosome structures has been based on so many fortuitous observations and preconceived ideas as that of the kinetochore. Even in the presence of overwhelming data, descriptions obtained in such a way are difficult to correct. Two examples of such a situation may be found in the papers by DAVIES (1956) and MONTEZUMA-DE-CARVALHO (1957).

DAVIES finds the kinetochore to be divided at metaphase of mitosis but he claims that this happens under abnormal conditions or after treatment. MONTEZUMA-DE-CARVALHO also accepts the evidence that the kinetochore is divided before metaphase of mitosis and metaphase II of meiosis but claims that the holding of the sister chromatids at the proximal regions of the arms is an artifact due to acetic-alcohol fixation. For him the chromatids are held together by specific attraction.

In both cases one is struck by the easy use of accidental observations and at the urge to perpetuate DARLINGTON's old interpretations. An inspection of MONTEZUMA-DE-CARVALHO's photographs shows clearly the matrix connecting the chromatids at the proximal regions of the arms, which is not represented

mitosis and already at metaphase I of meiosis invalidates DARLINGTON's theory on chromosome movements, since it was essentially based on the kinetochore's division cycle (LIMA-DE-FARIA, 1958).

The evidence that I gathered in recent years rules out the attribution of the described behaviour of the kinetochore and of the arms to abnormal conditions or fixation artifacts. These observations were based on the use of other fixatives than acetic-alcohol (such as Navashin—Karpechenko) and they were described in over 20 plants and animals living under normal conditions (LIMA-DE-FARIA, 1955, 1956, 1958). However, the decisive evidence on this question is found in observations made on living material.

BAJER and MOLE-BAJER (1956), and BAJER (1957) have recently made films of living chromosomes from the endosperm of *Haemanthus Katharinae* and other species. In these films, it can be seen with the utmost distinctness that the kinetochores are divided before metaphase of mitosis and that the sister chromatids are held together at the proximal regions of the arms by the matrix. Dr. A. BAJER has kindly put at my disposal some of his photographs from this material (Fig. 1). In addition, the kinetochore structure described at metaphase I of meiosis, in fixed and stained material, can be seen equally well in the living pollen mother cells of *Tradescantia paludosa* (LIMA-DE-FARIA, 1958).

RIS (1957) has not found sufficiently convincing evidence for the existence of a chromosome matrix. I have until recently taken a similar attitude. However, the behaviour of the proximal regions both in fixed and living material is evidence of its existence. From mitotic metaphase through preparation for anaphase, the sister chromatids are completely individualized; only a substance or structure holds them together until their final separation. This substance contains DNA as demonstrated by the application of the Feulgen test to the chromosomes of *Tradescantia paludosa*.

My study of living chromosomes of the staminal hairs of *Tradescantia paludosa* shows that the matrix is involved in holding together the sister chromatids throughout the whole length. The behaviour of the proximal regions represents only a delay in separation at a specific segment. The matrix surrounds the group of microfibrils which have been shown by electron microscope studies to constitute each chromatid (RIS, 1957) and allows chromatid separation by a kind of zipper system. The units involved correspond to the DNA elements which separate as a single block in tritiated thymidine experiments (TAYLOR, 1957).

Recent studies with the electron microscope confirm the existence of a structure or substance between the two chromosomes of a bivalent at prophase of meiosis (GIBBONS, 1958), and reveal a mass of dense material between the filaments that constitute the core of prophase meiotic chromosomes (MOSES, 1956). Whether we like to call this material matrix or something else is irrelevant. In all the cases mentioned we have a dense material or substance that is part of the chromosome but at the same time is distinct from the microfibrils, the filaments of the core, or the chromonema. Whether this material is a formation comparable to lampbrush extensions or a network of RNA and protein molecules surrounding DNA or a combination of both conditions, remains to be found out.

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Fig. 1. Untreated living cell of *Haemanthus Katharinae*. In the small chromosomes at one and nine o'clock the kinetochore may be seen divided and the matrix holding together the proximal regions of the arms.

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